

30 October 1980

Why bother to rehabilitate Galileo?

The Vatican has been edging towards a posthumous reconciliation with Galileo Galilei for several years. Since the time of Pope Paul VI in the mid-1960s, commendations of Galileo have been customary in the Vatican. The present pope, John-Paul II, has been still more outspoken. He marked the centenary of Einstein's birth two years ago with a public declaration that Galileo was an honoured precursor. As yet, however, the Vatican has not gone as far as to acknowledge as mistaken Galileo's prosecution in 1633 by the Holy Office — the trial that led to Galileo's abjuration of his heliocentric heresy on 22 June that year. Some such development is now, however, on the cards. The synod of bishops was told last week in Rome of a plan to set up a commission of inquiry under Archbishop Paul Poupard to look into the circumstances of the trial of Galileo and, as they say in the civil service, "to report". It is unthinkable that the outcome can fall short of a public declaration that Galileo should not have been brought to trial, that he should not have been forced by the threat of torture to recant and that his book, *Dialogue of the Two Great World Systems*, might safely have been taken off the Vatican's list of prohibited books a little earlier than 1835.

No doubt the Church is hoping that it will then be able to deal straightforwardly with the criticisms that have been flung at it for the past three centuries. Doctrinally, it should not be too hard for the commission to reach such a position. By good fortune, Pope Urban VIII (once a friend of Galileo's) played no part in the proceedings in Rome in 1633, not so much by design as from indolence. So a re-examination of the history of this shabby affair will not undermine the notion that popes (even bad popes) are infallible. Indeed, with a little luck, the commission may be able to put the blame for Galileo's examination and forced recantation on the two Dominicans, Badino Nores and Augustino Mongardo, who kept the minutes of Galileo's first formal encounter with the Holy Office in 1616, and who appear to have left in the Vatican files a much sharper version of what was then asked of Galileo than he and even his inquisitor, Cardinal Bellarmine, afterwards recalled. In the event, in 1633, Galileo was hauled over the coals not merely for having published his great book but also for having broken his supposed earlier undertaking not to hold heliocentric views. As the past three centuries have amply shown, this would not have been the first or last occasion on which the minutes of an important meeting turned out to reflect more accurately the wishes of the minute-taker than the words spoken.

Certainly, if it chooses, the commission will also be able to convince itself that the root of the trouble with Galileo lay in a misunderstanding between him and the Holy Office about the terms in which Copernicans were allowed to describe their theories. From 1616 on, Galileo understood that the Church required of him merely that the Copernican theory should be advanced only as a "hypothesis", not described in any sense as the "truth". Galileo, in other words, was almost as free to talk about Copernicanism as would be a modern professional brought up in the spirit of Karl Popper. Is not a good hypothesis one that is by definition falsifiable? That was the licence that he indulged in the seventeen years between his first and second encounters with the Holy Office.

The commission will also conveniently discover that Galileo's own behaviour contributed to the dismal outcome. Although his reputation is chiefly that of a fierce controversialist, Galileo was also capable of extraordinary deviousness. Why else would he have written the *Dialogue* as a dialogue between a Ptolemaic and a Copernican, leaving it to his readers to decide to which to give the

benefit of the doubt? Throughout his second trial, he lied consistently, denying Copernican inclinations. His final humiliation was his claim that the *Dialogue* had been written to demonstrate that Copernicus was wrong. Pathetically, he told the Holy Office that, in preparation for his examination, he had read the book again after an interval of three years and had been dismayed to discover that his literary intentions had gone awry: on reflection, he allowed, innocent readers might have been given the impression that Ptolemy and not Copernicus was mistaken. The cardinals of the Holy Office can hardly have believed their good fortune. For practical purposes, the trial was over before it had begun. Everybody present must have recognized that Galileo's repudiation of his own witty and incisive book would be a more serious penalty than any they could have dreamed up for him. Willingly, they accepted his lies at face value. The public confession he was required to read before being sent off to house imprisonment was almost a formality. The Holy Office had pulled off a huge success. To have made a heretic recant would have been a minor triumph, an everyday occurrence. To have so humiliated the source of an important heresy that it would afterwards be entirely discredited was a much greater victory.

Now, three centuries after the event, the new commission will be able to point out that Galileo was apparently a willing accomplice in the whole affair. The transcript of the proceedings is full enough for that to be plain. No doubt he was afraid, but does fear not make lying excusable? Indeed, the new commission may be able to proclaim, the events of 1633 might have taken a different course if only Galileo had been straightforward, sticking to his guns. He might even have been able to persuade his judges that the Earth goes round the Sun and not the Sun around the Earth. For much of his life, Galileo had indeed been trying to do just that. His first brush with the Holy Office in 1616 was provoked by a letter he wrote three years earlier in which he noted that "the holy scriptures cannot err" but went on to argue that over-literal interpretations of them might be misleading. In the last resort, he had found out, the Church, not he, held the master cards. The execution of Giordano Bruno in 1600 for a similar (if less cogent) proclamation of heresy would also have shown Galileo that on some important issues the Church was implacable. If Galileo had stuck to his heresy, the chances are high that even he would have followed Bruno to the stake. Then, the Poupard commission would have a much more difficult circle to square.

So why, if the humiliation of Galileo falls short of martyrdom, is it now thought necessary to reopen this old question? What the synod of bishops was told last week is that the trouble about Galileo still serves as a barrier between science and the Church, keeping potential believers away from the congregations to which they would otherwise belong. It is hard to think this can be a substantial consideration. Even those who have no allegiance to the Catholic Church now acknowledge that there have been substantial changes in the past three centuries. Willy-nilly, the old literal interpretation of the Bible has been watered down. Many distinguished clerics take a serious and constructive interest in what is happening in science. Many distinguished scientists are also believers. And if there is a gulf between science and the Church, its origin lies in contemporary issues, not those with which the seventeenth century was preoccupied.

That, indeed, is the reason why the new commission may have a harder task than its planners appear to be expecting. With the passage of all this time, the rehabilitation of Galileo is hardly more significant than the posthumous pardoning of a traffic



violator who pleaded guilty in court. The new commission cannot expect to produce a persuasive report on Galileo without going thoroughly into the question of how the Church should make up its mind about scientific issues which are at present contentious. How will the Church make up its mind about Darwinism, for example? Hankerings after directed evolution still play too big a part in the Church's view of biology. What will happen if the Big Bang turns out not to be the cosmological convenience that the Church has leaped at? It is tempting to suppose that in these enlightened days the tragedy of Galileo could not be repeated. But is that so? What is to be made of the way in which the Church, by

insisting on its views of how people should behave, frequently flies in the face of what is known about human behaviour? Only last Sunday, Pope John-Paul II, a charismatic publicist but an illiberal pope, was asking that remarried people should refrain indefinitely from sexual intercourse. The Church's views on birth-control, appropriate enough when the chances of newborn children reaching puberty were a good deal less than fifty per cent, are now a means of making millions small-time martyrs, pathetic contemporary analogues of Galileo. The Poupard commission will steer away from cans of worms like these. In doing so, it will fall far short of what seem to be its objectives.

Solutions for transatlantic universities?

Everybody agrees that higher education is important. Many people spend time worrying about its health. Almost nothing is being done. This, it seems, is the lesson to be drawn from the clutch of reports on higher education published in the past few days. The meeting organized by the European Community at Strasbourg last week (see page 773) was presented with a familiar analysis of what the future holds (see *Nature*, 23 October); immobile university teaching staffs unlikely to be renewed on a substantial scale for years ahead, indefinitely straitened budgets and declining student numbers. What the commission's working paper did was to extend to the whole of Europe the gloomy analysis of the future for British universities described just over two years ago when Mrs Shirley Williams was Secretary of State for Education and Science. According to temperament, some academics (and some of their well-wishers) will be heartened to know that misfortune is so widespread while others will be dismayed that the problem is so huge.

Yet the problem is also unchanging, or apparently so. This week's report from the House of Commons Select Committee on Education (see page 770) makes that plain. The committee is still urging action on issues that first became urgent years ago. Has the dual support system for research quite broken down, and if so why? Can something be done to make the Universities Grants Committee a better buffer between the universities and central government? What should be done to help universities deal with the inflexibility that demography has thrust upon them? The committee has some sensible and even original things to say. Whether they will evoke a response from government departments whose present concern is what it seems continuously to have been for the past several years — to find further economies in public spending — is another matter.

Only in the United States does it seem that optimism survives — and even that may be illusory. The report on science and engineering education put together for the White House by the National Science Foundation and the Department of Education (see page 769) is no doubt right to deny some of the wilder fears about the adequacy of higher education in the United States — the charge that the efficacy of United States defence may be jeopardized by the quality of graduates from the universities for example. Moreover, there is no substance in the wish that some sleight of hand by university administrators might of itself beat back the tide of imports from Japan and elsewhere. If the United States is slipping as an economic power, it is largely because its people have elected to enjoy more of the benefits of service industries than the people of states such as Japan (see *Nature*, 2 October). The White House report nevertheless discovers a number of points at which the university system is under stress. The difficulty of persuading engineers to teach other engineers is not an isolated problem but a sign that universities may chronically be least able to recruit the teachers whose services are most needed. And if the terms of reference of the study had been wider, it would probably have come to conclusions that echoed, perhaps less starkly, those of European commentators — ageing teaching staffs, too much immobility among them and the prospect of too few students. What on earth is to be done?

Times like the present, when most governments are

preoccupied with serious financial problems, are not propitious for grand designs for reform. Radical and sweeping change is easiest when funds are plentiful. The most serious danger in the prolongation of present difficulties, however, is that university systems on both sides of the Atlantic will be further impoverished in their present pattern until change heralded by crisis is forced upon them. This is why, while waiting for the grand design, some of the common problems of these several university systems need to be tackled piecemeal and empirically.

The question of academic tenure is an obvious problem, with which American universities have grappled for several years. The House of Commons committee deals with the issue judiciously. Tenure is an essential ingredient of academic freedom, but there is no reason why a university that no longer needs a whole department should not invite the teachers concerned to leave, on terms regarded by their academic colleagues as equitable or even generous. Most European academics will resist this notion, familiar though it has unhappily become in the United States. Yet if the choice is between the health of a university and the discomfort of some of its academics, can lifelong tenure be justly held always to be beneficent? European universities are mostly too mealy-mouthed on this delicate subject — and too free with early tenure in any case.

Shortages of teachers in specialized subjects, identified in engineering by the White House report, are commonplace and also chronic. They are, however, by no means as novel as they seem — both in medicine and the law, most university systems have special devices for persuading high-earning professional people to devote themselves partly to the interests of students, their future competitors. In more novel fields, systems engineering for example, there has not been — and probably will not be — time for suitable inducements to be devised. Universities may have to manage indefinitely with unconventional solutions. Part-time teachers drafted in from industry may help but will not solve all the problems that abound. The general solution must however be more flexible and permanent. Is it not time that universities hard-pressed to teach students what only industries know and practise should, without shame, arrange to have their students taught vocational skills on the job?

Another conspicuous element of the common malaise is that universities are no longer as autonomous as they like corporately to pretend. Especially in Europe but also in parts of the United States system, external agencies have an increasing say in the development of policy. The explanation is simple — governments pay an increasing share of the costs, and expect an increased influence. Yet governments, however liberal, are less able to know what are the needs of individual universities than are the academics who work in them. In principle, academics are also best placed to judge their contribution to national needs, and their places in the university systems to which they belong. The precondition, however, is autonomy — the freedom to make decisions for themselves. In this sense, the demand that universities should be free from some of the shackles that at present bind them is not a demand for the right to irresponsibility but the opposite. More autonomy is certain to mean better universities.

More commercial genetic manipulation

Harvard and Biogen establish Cambridge labs

Washington

Not to be outdone by West Coast colleagues, scientists from Harvard University and the Massachusetts Institute of Technology (MIT) are busy with their own plans for the commercial exploitation of research using recombinant DNA techniques.

The president of Harvard, Dr Derek Bok, is expected to announce shortly the university's decision to become a shareholder (although not an investor) in a company being set up to develop gene cloning techniques evolved in the university's laboratories. One candidate technique is Dr Mark Ptashne's methods of cloning human fibroblast interferon genes in *Escherichia coli*, reported in the latest issue of *Proc. natn. Acad. Sci. USA* (77, 5230; 1980).

At the same time, Biogen, the Swiss-based genetic engineering company whose board of directors includes Dr Walter Gilbert of Harvard and Dr Philip Sharp of MIT, is seeking permission to construct a \$5.5 million research and manufacturing facility in East Cambridge.

Both schemes would make it easier for senior research scientists to maintain their university positions while working as consultants for the companies. In the case of Harvard — which 2 years ago agreed to license the rights to Dr Gilbert's insulin research to Biogen — the university would be able to invest its share of the profits from the new company in further research.

Inevitably, both plans have caused controversy. Harvard faculty members have been concerned about the potential conflict of interests between the goals of the proposed company and the university's commitment to basic research; Biogen's plans were the subject of a public meeting held on Tuesday before the Cambridge Biohazard Committee to discuss whether any conditions should be placed on the company's activities.

The Harvard plan was the brain-child of Dr Mark Ptashne, professor of biochemistry and molecular biology. Initially it had been proposed that the company would, at least temporarily, rent space in the university's biology laboratories; that postgraduate students might be able to work for the company while retaining university appointments; and that finance would be largely provided by the pharmaceutical company Eli Lilly, which had earlier bid unsuccessfully for the licence to Dr Gilbert's research.

Following an intense debate over the

summer, various steps have been taken to keep the company, as one university official describes it, "very much at arm's length". For example, it has been agreed that, even if the university's involvement is approved, the company will seek separate premises.

In addition, although some Harvard scientists would be shareholders in the company and could be employed on a one-day-a-week basis as consultants — the generally accepted limit to outside commitments — direct overlap with university research staff would be minimal.

Financial support would probably come from outside sources of venture capital under a scheme being worked on by the Harvard Management Company, which manages the university's endowment.

The proposed organization of the company and its relationship to the university was discussed last week at a meeting of the faculty of arts and sciences. Although no formal decision was taken, Mr Daniel Steiner, Harvard's general counsel, said after the meeting that he saw "no overwhelming obstacles" to the plan being approved by Dr Bok, adding that the university "would not have gone as far as we have" if it had not been seriously interested in the project.

Ironically, the faculty meeting took place the day after Biogen had told the city council in Cambridge that it wished to build a research and manufacturing facility in the city. At present, Biogen's main research facilities are in Geneva; and it is rumoured that one of the reasons for

planning to build the new facility in Cambridge is the difficulty that has been experienced in obtaining Swiss work permits for US postdoctoral students.

Biogen's proposal was due to be presented on Tuesday to the Cambridge City Biohazard Committee, a subcommittee of the City Health Policy Council set up in 1976 after an intense local debate to monitor recombinant DNA research in the area.

More criticism was expected at the meeting. Ex-mayor Alfred E. Vellucci, who previously led an unsuccessful fight to ban all recombinant DNA research in Cambridge, was equally opposed to a "DNA factory" coming to Cambridge now.

Others thought that — particularly in the light of the investment fever that swept Wall Street two weeks ago over the public offer of shares in the San Francisco company Genentech — Biogen is unlikely to encounter substantial opposition.

If the Biogen facility gets the go-ahead, it is likely to be partly financed from a \$20 million equity investment which the chemical company Monsanto is to make in the company. Also, the two other major equity holders — Shering Plough and International Nickel — have increased their equity by \$8.8 million.

In a separate development, the Dow Chemical Company announced that it was making a \$5 million investment in another small Massachusetts biotechnology company, Collaborative Research, of which Dr David Baltimore is chairman.

David Dickson

Few complaints on education

Washington

US science and engineering efforts are basically on an even keel, even if there are a few selective vacancies among the crew, the officers do not understand too much about how the engines work and the passengers occasionally question the course that is being followed.

This seems to be the main message of a long-awaited report on the state of US science and engineering education, requested by President Carter in February from the National Science Foundation and the Department of Education, and published in Washington last week.

The request, largely prompted by the President's Science Advisor, Dr Frank Press, responded to certain concerns expressed about aspects of educational policy in these two fields. These include a well documented decline in performance by US school-children in science-related subjects, difficulties faced by the armed forces in retaining technically trained recruits (much talked about after the failure of the attempt to rescue the hostages from Iran) and warnings of the dire consequences of apparent Soviet educa-

tional supremacy.

But whereas in the mid-1950s the launching of the Russian Sputnik was seen as a challenge which led to a massive injection of new funds to revitalize US efforts to improve scientific and technical skills, the new report implies that there is little need for general alarm — or any major investment of resources.

This time, the emphasis is on quality rather than quantity. The report concludes that the number of science and engineering graduates is likely to be adequate for the next couple of decades, apart from possible shortages in fields such as computer science.

Two main problems are identified: first, the increasing cost of providing a good engineering education, both in terms of employing good teachers and purchasing up-to-date equipment, and, second, evidence of a spreading "scientific illiteracy" which could have serious consequences in a world of increasing technical complexity.

It is the latter problem which provokes some of the strongest language in the report, with the claim that there is a

"desperate need" for curricula for students who are not interested in professional scientific and engineering courses, and that "the current trend toward virtual scientific and technological illiteracy, unless reversed, means that important national decisions involving science and technology will be made increasingly on the basis of ignorance and misunderstanding".

The evidence used to support this contention — headlined in the US press coverage of the report — is scarce. However, various background studies are quoted. These reveal, for example, that in 1976–77 about 48 per cent of grade 9–12 students were enrolled in at least one science class. This compares with more than 59 per cent in 1960–61, the crest of the post-Sputnik science education boom.

Among the proposals aimed at tackling the growing gap between the two cultures is the setting up of a President's Council on Excellence in Science and Technology Education, to act as a possible vehicle for presidential statements about the need to raise science and mathematical requirements for all secondary school students. Other suggestions include a federal inter-agency coordinating committee of public understanding of science and technology, various regional conferences to help state and local administrators to discuss with teachers and educationalists ways of improving the quality and attractiveness of science courses, and various measures to increase awareness of the need to prepare for career opportunities in science and technology.

On the question of whether the current supply of science and engineering graduates is sufficient, the report is reassuring. In terms of scientists, a background "staff analysis" says that the present supply is adequate to satisfy demand except in a few sub-fields of physical and biological science, and that in 1990 the aggregate number of new science graduates at all degree levels should exceed the number able to find jobs in the broad fields in which they are trained.

In engineering, the picture is less optimistic. The same analysis indicates that there are current shortages of trained computer professionals and most types of engineers at all degree levels, and it says that for computer professionals in particular a shortage at all degree levels is likely to persist beyond 1990.

The general conclusion, however, is that the numbers of new engineering baccalaureates should be adequate to satisfy demand for the services, with neither a rapidly increasing defence budget nor a major programme in synthetic fuels creating significant additional demands.

Where the report does pinpoint a cause for concern is in the supply of engineering teachers and equipment. In the first case, it suggests that increased support for university-based research projects, and possibly salary increases for researchers

approved by separate federal agencies, could reduce the rewards gap between industrial and academic careers.

In the second case, officials from the Office of Science and Technology Policy point out that the upgrading of university equipment is a principal objective of the extra \$600 million in basic science support which President Carter pledged in his economic revitalization package announced in August.

The report's proposals are now being debated inside the White House to find those which, at a time of general budget austerity, can still be squeezed into the budget request for the fiscal year 1982. In a covering letter to the report, Dr Frank Press says that the Administration intends "to enhance the capabilities of our science and engineering programs for education and research".

However, following a report which throws lukewarm water on some of the more extreme claims and fears that have been expressed over the past year — nothing very dramatic is expected.

David Dickson

UK higher education

Commons take stock

British universities have at last found some friends in the House of Commons, to judge from the report (published earlier this week) of the inquiry by the Education, Science and Arts Committee into the funding and organization of higher education. Specifically, the committee sternly rejects the implications of the question raised by the Secretary of State for Education and Science, Mr Mark Carlisle, that the pattern of university courses should be tailored to the forecast needs of skilled manpower in the United Kingdom.

The committee has also sided with the universities in its urging that steps should be taken to encourage more potential students to go on to higher education, thus denying Mr Carlisle's statement that the present participation rate reflects market forces and should not be "artificially boosted". The question of participation rates is crucial for the universities, faced as they are with the prospect of a declining demand for university places for demographic reasons.

On the administration of institutions of higher education other than the universities (polytechnics, technical colleges and the like), the committee does not go the whole hog with radical opinion and recommend a committee analogous to the University Grants committee for channelling funds in their direction. Rather, it advocates a committee to be responsible for planning and advice in the non-university sector, saying that the local connections that have grown up between local authorities and the institutions which they maintain are valuable.

The committee links its opinion on the participation rate with the financial

problems of higher education. It describes as "disappointing" Mr Carlisle's explanation of what is meant by "level funding" — the procedure by which the universities' budgets for the two coming years will "not be very different in real terms" from that for the present academic year. The committee says that the policy of "level funding" leaves no room for potential increases in the age participation rate, and that the government should instead devise a formula relating expenditure to student numbers.

The committee's approach to Mr Carlisle's delphic hope that there would be a "subject balance in broad terms in relation to the future of highly qualified manpower" was first to take advice on its meaning from his officials. For its pains, it won the supposed clarification that the objective was to translate "the very specific requirements of the market-place into very broad subject areas, grouping them in, for example, the technologies, the sciences and the arts subjects . . . to try to work out from such messages as can be derived . . . a subject profile which could be a guiding principle for the institutions of higher education".

While aspects of this opinion were echoed by others among the committee's witnesses, the committee says it found no agreement on what might be called the national need, and no workable suggestions as to how the task might be accomplished were put forward. The committee therefore recommends that the universities should indeed listen to such "messages" as they can hear, but that the suggestion that they should then attempt to work out "principles for higher education" should be rejected as impracticable.

The committee also asks that the University Grants Committee should be made more independent of the Department of Education and Science, perhaps by recruiting its own officials, not borrowing them from the department. It applauds the setting up of the Merrison committee, intended to look into the joint support of the University Grants Committee and the research councils for university research, and approves of the consideration now being given to the concentration of resources in those establishments where research is most vigorous.

For the rest, the committee gives its blessing to the department's present investigation of student loans, asks for a better definition of the problems of different fees for home and overseas students and suggests that the concept of tenure should be re-examined. The committee is judicious on the last point. It acknowledges that tenure is an important ingredient of academic freedom, but asks that it should not stand in the way of change. One solution, the committee suggests, would be for academics displaced from unwanted departments to be offered "fair redundancy".

European Space Agency

Supposing stability

The European Space Agency (ESA) is hoping for a budget held steady for a decade, at a real £285 million a year from 1982. The sum is much less than ESA's current budget of £480 million, but it sets a floor below which ESA's rapidly falling spending should not fall.

This comes at a time when all is changing at ESA under Erik Quistgaard, the 58-year-old Danish industrialist who was appointed director-general of ESA in May. The current troubles come from the ending of the development programme for Ariane, ESA's launcher, and its transfer to the private company Arianespace, and the completion of Spacelab, due for launch on the space shuttle in 1983.

Last week Quistgaard presented his proposals to the ESA Council — the top decision-making body of ESA — in an unminuted restricted session of the Council "bureau", which consists of the top national delegates but takes soundings rather than decisions. It was this bureau session which agreed in principle to Quistgaard's budget. The bureau also supported his proposals for a 50 per cent increase over the next ten years in the mandatory science budget (which provides ESA's research satellites — 12 launched so far). This budget has been fixed effectively since 1971 at a level of £60 million a year (1980 prices), and is committed — all but £180 million — to 1990 on six projects.

These are a trip to Halley's comet in 1986 (a mission called Giotto), an astrometry satellite (Hipparcus), an X-ray observatory (Exosat), a 15 per cent involvement in the space telescope, an investigation of the solar corona outside the elliptic, and an experiment to measure the effects on the human body of controlled accelerations in space (SLED).

Whether the money materializes depends on decisions made nationally, and at subsequent meetings of ESA council (the next is this month). Agreement must be reached on the main technological programme of ESA — is it to be improvement of Ariane, Earth resources or communications satellites — against a tendency in France and Germany, opposed by smaller nations, to see ESA primarily as a research and development agency rather than a profit-making space multinational. And there are problems in both France and the United Kingdom over the procedure of contributing to ESA; so a ministerial meeting is being considered for the spring of next year.

In the United Kingdom, the problem is the division of contributions between the Department of Industry (for applications) and the Science Research Council (for research), so that a change in balance must be decided at ministerial level. In France, the problem is the competition between contributions to ESA and a growing

national programme within the budget of a single agency, the Centre National pour les Etudes Spatiales — again a matter for cabinet-level decisions.

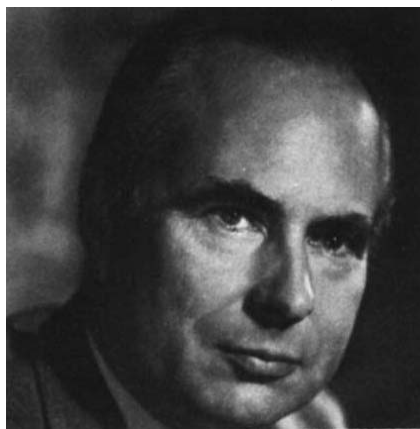
But whatever the remaining problems, Quistgaard himself scored a major personal success last week. Delegates came to Council prepared to take their pound of flesh — France, for example, wanted a 10 per cent cut in ESA staff — but in the end they applauded him. In his summing up Quistgaard told delegates "You nearly killed me — but I survived." More than that, he has streamlined ESA, getting Council to agree to eliminate the 40-strong directorate of future programmes and planning, and to set up a think tank of half the size which will report directly to Quistgaard; and to devolve power to the existing directors (now to be called the management team).

Robert Walgate

Huxley for PRS

Sir Andrew Huxley, the neurophysiologist, is now almost certain to be the next President of the Royal Society in succession to Lord Todd, who comes to the end of his five-year stint at the anniversary meeting, to be held this year on 1 December.

The society's election procedures require that nominations of new council members and also of the new president should first be agreed by the Council, and then submitted to the membership of the society in the form of a single slate of candidates. Sir Andrew Huxley's name



has apparently been agreed for several months, and it is known that several institutions have already engaged him to speak at conferences or to officiate at centenary celebrations well into 1981.

Now that Sir Andrew's nomination by the council is assured, the chances of his not being elected are vanishingly small. This could only happen if a majority of the members of the society were to strike out his name on the ballot paper and to substitute that of some other member.

Sir Andrew, who will be 63 at his election, has been a Royal Society Research Professor in the Department of Physiology, University College London, since 1969.

Indian laboratories

Back to GO*Bangalore*

In what is considered to be a major step to tone up the state of Indian research, the government led by Mrs Gandhi has transferred back to the Council of Scientific and Industrial Research (CSIR) four laboratories which the previous Janata government had handed over to user ministries. The laboratories concerned are the Central Fuel Research Institute, Dhanabad; the Indian Institute of Petroleum, Dehra Dun; the Central Road Research Institute, New Delhi; and the Central Building Research Institute, Roorkee.

In April 1978, the Janata government, which under Morarji Desai was firm on "socially committed research", had dissociated these laboratories from CSIR and transferred them to user ministries on an experimental basis. Presumably this "delinking and transfer" was intended to foster a closer relationship between research and industry that would accelerate the country's scientific potential for industrial growth.

However, the decision proved to be counterproductive. Not only did it fail to achieve the desired goal but India's scientific community saw this step as a "vicious attack on scientific autonomy". For the past two years there has been a heated debate on the move in India's administrative and political circles. It was widely alleged that the decision was taken without considering the opinion of the scientific community.

The controversy surrounding the delinking had cast a shadow over scientific research in India. While the advocates of delinking called it an important measure to enhance industry — "research interaction" — the critics saw it as "a dark conspiracy of alien agents acting in collusion with their Indian masters" to subvert indigenous research efforts.

CSIR was established in the early 1950s to promote scientific research into the economic exploitation of India's vast resources. By Indian standards, CSIR is a giant covering four laboratories and research associations, and it has never been handicapped by the paucity of resources or dearth of skilled manpower.

The contribution that the CSIR laboratories have made to indigenous research for industrial development is difficult to assess. While the left-inclined high-technology advocates say that CSIR has been a trail blazer in easing India's poverty and backwardness through innovative research relevant to the socio-economic conditions of the country, pro-Gandhian, appropriate-technology proponents say that CSIR is a "colonial set-up subservient to the capitalistic research" that is alien to the Indian environment.

In recent years, CSIR technocrats have

been complaining that there are virtually no takers for the processes and technologies developed in CSIR laboratories. In fact, most public and private sector industries in India have favoured the wholesale import of foreign technology. This anomaly was said to be the motivation behind the handing over of the laboratories to user ministries. But the import of technology by the bureaucrats in the user ministries has continued unabated.

B. Radhakrishna Rao

NIH research grants

Trying new tricks

Washington

In an attempt to cut down on the amount of "time and effort" reporting required of scientists, the National Institutes of Health (NIH) are being urged to try a new mechanism for funding research grants that would make the researcher financially accountable to his or her university or research institution rather than directly to the funding agency.

At present, NIH project grants — whose total value is about \$1,400 million, over half the total NIH research budget — are awarded on a "cost reimbursement" basis, under which the government agrees to cover all previously agreed costs that can be properly accounted for.

The proposal is to experiment with so-called "fixed obligation grants" (or "fixed price grants") where the research institution merely has to demonstrate to the funding agency that the scientific and technical goals of the research have been satisfactorily pursued.

"Time and effort" reporting is the most controversial of the strict new rules on accounting for research expenditures introduced last month by the Office of Management and Budget (OMB) in a document on cost principles known as Circular A-21.

These rules require all principal investigators to provide a semester-by-semester breakdown of the way they distribute their time between teaching, research, administration and other activities — and to report any change in this distribution to the federal government.

Federal auditors argue that this is necessary to ensure that money is being allocated and spent in the way agreed when a research grant is awarded. But scientists argue that in an over-zealous enthusiasm to minimize fraud and abuse — a popular target of congressional committees — the auditors are reducing the productivity of research laboratories for a minimal return.

Despite earlier protests, OMB had refused to delay the implementation of the new rules, the outcome of several years of negotiation. However, under continued pressure from universities, the agency is now prepared to discuss ways of reaching its accountability goals more effectively.

OMB has already agreed to experiment

at ten universities where "time and effort" reporting will be on a statistical basis rather than professor-by-professor. What is now being suggested, however, is considered by NIH director Dr Donald Frederickson to be revolutionary — "as radical as *Finnegan's Wake*".

The outlines of the proposal were presented to the NIH Directors' Advisory Committee (DAC) by Dr Linda Wilson, Associate Vice-Chancellor for Research at the University of Illinois in Urbana, and Mr James Kelly, previously Executive Vice-Chancellor of the State University of New York and a long-time proponent of fixed-price contracts.

The general idea is that there would be no change in the present pre-award proposal process for the selection of research and determining the amount of an award. However, post-award administration would be changed to delegate most of the responsibility to the recipient institution and the principal investigator, in particular the emphasis of accountability would be shifted from the allowability of costs and the adequacy of documentation to criteria that indicate "reasonableness of technical progress".

Supporters of this new proposal, which comes out of a recommendation made in a recent report from the National Commission on Research for Experiments in Grant-in-Aid Support for Research Institutions, argue that it should still be possible to build in enough controls to ensure that public funds are not misused (such as spot auditing checks). The new system might eliminate some existing problems, but there could be new ones. For example, by shifting prime responsibility for the financial conduct of the grant from the federal government to the research institution, tensions between the government and the institutions could be replaced by tensions between the institution and its principal investigators.

Additional pressure would also be incurred on efforts to measure scientific accountability; Dr Wilson emphasizes that research grants should be treated as *assistance* rather than *procurement* funds, to avoid the rigid accountability — and hence loss of flexibility.

Mr Kelly told members of the DAC that there was little evidence that a new system for administering grants would save much money and that any increase in research productivity would not necessarily be measurable. The principal advantage, he said, was that the new approach might reduce tensions between universities and the federal government, currently running high in the wake of the introduction of Circular A-21.

Any experiments in this direction are likely to receive the approval of the Office of Science and Technology Policy (OSTP), whose associate director, Dr Denis Prager, told the committee that reducing non-budgetary constraints on research was one of OSTP's top priorities, particularly by

encouraging forms of regulation based on performance.

Polish academy

Flexing muscle

Polish scientists wishing to travel abroad for professional purposes should in future find it considerably less complicated to obtain the necessary passport. Last week, Dr Jan Kaczmarek, Academic Secretary of the Polish Academy of Sciences, announced that the academy now has the right to decide such matters for its members.

This announcement marks a small, but significant step towards the greater academic autonomy widely demanded by Polish intellectuals in the wake of the Gdansk accords. It was made at an extraordinary general meeting of the Polish Academy of Sciences, which was called to discuss and re-evaluate the role of the academy, and of the scientific establishment generally, in the light of the recent changes in the country. The meeting, which participants reported had a warm and open atmosphere, made some sharp criticisms about the situation in Poland during the past few years, in particular, both the over-centralistic attitude of the authorities, which made it extremely difficult to get a

No stay for badgers



This will be a bad week for British badgers. Today (Thursday, 30 October) Lord Zuckerman's report on the practice of gassing badgers thought to be infected with bovine tuberculosis will be made public. This issue is contentious among conservationists because the Badger Act, carried through the British parliament with some emotion, which makes it a criminal offence to kill badgers even when they damage land and crops, provided an exemption for the Ministry of Agriculture, Fisheries and Food to allow the destruction of badger hides thought to be a reservoir of bovine tuberculosis.

Conservationists have since protested that the practice of gassing badgers for the sake of protecting cattle has been too widely licensed, and that it is in any case unnecessary or ineffective. Lord Zuckerman's report it thought to argue in an opposite direction.

hearing for constructive criticism, and the consequent lack of responsibility. To remedy this situation, the academy called for clear limits to be set to the competence of anyone in authority and of rank-and-file functionaries.

A comprehensive system, the academy said, must be worked out for the proper functioning of the economy, based on the "broad utilization of all available and permissible self-regulating mechanisms". The authorities, it said, must show they trust the people. One obvious sign of such trust would be freedom of expression and the phasing out of censorship (except for the minimum needs of national security), which constituted a major point in the Gdansk accords.

A prominent speaker on this theme was Dr Jan Kielanowski, a leading nutritionist. He reminded the meeting that he and Dr Edward Lipinski had tried to raise the same issue of academic freedom and the abolition of the censorship at the academy's Annual General Meeting in May 1979 — but on that occasion, the motion was ruled out of order from the chair. Now, however, he observed, when the initiative came not from a couple of members of the academy, but from the broad masses of the Polish workers, the academy was prepared to listen.

The academy, in fact, had not waited until the extraordinary general meeting to take up the issue. Censorship and academic freedom had already been raised at the praesidium of the academy on 30 September. On that occasion, Professor Janusz Groszkowski, a former chairman of the academy, called for a detailed analysis of the situation in Polish research, including the moral and professional scrutiny of those researchers and scientists known to be letting down the intellectual integrity of their profession. Some researchers, said Groszkowski, are guilty of plagiarism, intellectual dishonesty, idleness and ignorance, while young people of genuine talent were given no opportunity to develop freely. "Hence", he concluded, "we have no great names and no great achievements." The academy, he said, must take the initiative in the reform and renewal of the scientific profession, without waiting for a lead; its deliberations should include possible changes in the legislation governing scientific research, the management of scientific institutes and the question of independent, trades unions for scientific research workers.

However, before this last proposal could be put to the general meeting of the academy, Poland's scientists had made their own decision: at the second delegate meeting of the new "Free Trade Union of Scientific, Technical and Educational Workers" (13 October), it was decided to terminate the existence of this body as a separate entity, and to transform it into a branch of the "Solidarity" confederation.

Vera Rich

European community

Research without end

Strasbourg

A meeting called by the European Commission here last week ended with a general air of expectancy among its participants. Next June, the commission will be proposing to the Council of Ministers a new research "enterprise". So far, however, it is not known how much the enterprise will cost, when it will start or even what it will do.

Commission officials nevertheless consider that last week's conference gave them a mandate to do something. And whatever it is, the cost will be added to the commission's present research budget, running at five per cent of the member states' research and development budgets.

The impetus for the initiative is twofold. First, there is a feeling in Brussels that there will be some slack in the EEC budget in the near future. Some think the Common Agricultural Policy will collapse, others that some part of agricultural spending will be put to other uses. All the community's

directorates-general, including that for research, science and education, are eager to pick up the crumbs.

There is also a sense that something must be done to free posts and talent at the universities. As Sir Hermann Bondi put it last week, "the universities have been static for many years while other things are changing fast". He went on to advocate the setting up of research structures parallel to but in touch with the universities.

Most of those at Strasbourg agreed, but failed to provide the political wrapping or the precision that might lead to practical proposals to create scientific jobs or to improve mobility in Europe. In the end, the commission will work out its policy on the basis of background documents* prepared for Strasbourg, particularly those of Guy Denielou, president of the Technical University of Compiègne, Ilya Prigogine of the Free University of Brussels and Freddie Clarke, research director responsible for renewable energy at Harwell. Dr Günter Schuster, who heads the Brussels directorate-general for research, science and education, will now set up a task force to prepare proposals for June. There will be three scenarios, from ambitious to pessimistic, to cater for the unknown political climate nine months hence.

Schuster will rely largely on Denielou's proposals to define the "enterprise". But, says Denielou, the definition should concentrate on structure rather than topics. How the enterprise should function is more important, at this stage, than what it should do. The structure should not be academic, but like a business. It should be flexible, creating and closing teams as needs change. Its administration should be "very light". It should be cheap, probably based on existing laboratories.

A principal objective would be to foster mobility, for scientists are considered less free in Europe than in the United States. Research teams in the enterprise would therefore be drawn from across Europe. He proposes an experiment — three years, 50 teams of 30 scientists each, costs borne largely by the host laboratories.

To determine the subjects to be studied, the enterprise would need what Prigogine calls "an organ of perception" — something like the National Research Council in the United States — which would also select institutions and teams to investigate the chosen topics, using international referees. Brussels would thus be gaining an instrument for a centralized research policy — and a critical question for June will be what relationship such an instrument should have to the council, the supreme body in the community.

Robert Walgate

*"Towards a new undertaking in European research" by Guy Denielou and others; "Science and society in a changing Europe" by Ilya Prigogine; and "Community industrial research priorities for the early 1980s" by F.J.P. Clarke and others. (Commission of the European Communities (DG XII), Brussels.)

China plays profit

Researchers at the Institute of Physics of the Chinese Academy of Sciences have recently been working on a new project — how to sell their results to industry.

It seems that until the recent economic reforms, what is now officially described as "a lack of communications" and "obstacles inherent in the system" prevented new scientific results from being applied to industry. New findings remained inaccessibly locked away in publications and festschrifts. Consequently, scientists had become upset and frustrated, while factories, unaware of new advances, were unable to modernize.

With the fall of the Gang of Four, however, factory managements were able to approach the scientists for advice on new results and how to apply them. So far, the Institute of Physics has worked out three possible cooperation procedures: (1) outright sale of the new technology, with technical assistance provided until the technology goes into production; (2) contracts for technological guidance, involving sending experts to the factories or training workers at the institute; with the onset of production, the institute is repaid on a sliding scale related to the profits from the new technology; for example, 20% in the first year, 10% in the second, 5% in the third; (3) provision of the new technology, for a decreasing percentage of its profits, for example, 5% the first year, 3% the second, and so on.

So far, the institute has signed contracts with 19 factories, many of which are already benefiting financially from such deals.

Vera Rich

Dangerous pathogens

New UK committee

The long-awaited reorganization of the British mechanism for regulating research with dangerous pathogens is planned for early in 1981. Last week the Department of Health and the Health and Safety Executive made public the terms of reference of a new regulatory body, to be known as the Advisory Committee on Dangerous Pathogens. The chairman and members of the group, which will replace the Dangerous Pathogens Advisory Group, have yet to be appointed.

The days of the existing advisory group have been numbered since the smallpox accident at the University of Birmingham in August 1978. Set up in 1975, the group was first responsible for drawing up lists of pathogenic organisms supposed to involve various degrees of hazard. The group also advised the Department of Health which laboratories should be licensed to work with organisms in the most hazardous category, category A. Control was voluntary.

The new system will be backed by the statutory requirement that laboratories planning to work with dangerous pathogens should first notify the Health and Safety Executive (HSE) of the people who will be involved and the experiments planned, giving at least 30 days' notice. The draft regulations published by HSE require more cursory notification of other listed pathogens.

Under the proposed arrangements, the new advisory committee will not scrutinize safety arrangements in individual laboratories, which will in future be the responsibility of officials of HSE. Instead, it will be responsible for advising the executive as well as the ministries responsible for health and agriculture on the classification of pathogens, the development of safety procedures and the opportunities for research.

One of the peculiar difficulties of the regulation of dangerous pathogens affects the working of medical diagnostic laboratories, which cannot know in advance whether a corpse on the slab has been laid low by, say, lassa fever. The proposed notification regulations will not apply to such laboratories, which will nevertheless be required not to carry out

Schedule 1 pathogens

Crimean haemorrhagic fever virus (Congo)
Ebola virus
Jumin haemorrhagic fever virus
Lassa fever virus
Machupo haemorrhagic fever virus
Marburg virus
Rabies virus
Simian herpes B virus
Smallpox virus
Venezuelan encephalitis virus

further work with schedule 1 organisms without going through the full procedure.

The composition of the new advisory group has been settled only in outline. There will be five employers' representatives drawn from universities, public health laboratories and the like, five representatives of employees' organisations and ten scientific members, together with a chairman.

For researchers in British laboratories, the chief consequences of the proposed arrangement will be that in future control will have the force of law. Failure to notify will be an offence, as will be failure to follow approved codes of practice.

Soviet geophysics

Drilling deeper

Soviet geophysicists have had to rethink their models of the deep strata beneath the European part of the USSR, after processing the preliminary data from the super-deep borehole now being drilled on the Kola peninsula. And, according to Professor Vladimir V. Belousov, head of the Scientific Council on the Earth's Crust and Upper Mantle of the Department of Earth Sciences of the Soviet Academy of Sciences, two and possibly three more such holes will be started in the near future.

The Kola bore can advance at a rate of some 10–12 m per day, and has now passed a depth of 10,000 m. It is sited in an area thought to be geologically "dead", and without risk of earthquakes or volcanic activity. Core samples, however, have revealed that cracking extends to considerable depths, and at these levels, at a temperature of about 150°C, significant quantities of aqueous solutions, carbon dioxide and helium provide evidence of high geological activity.

This remarkable level of activity is not the only surprising result to have come from the Kola bore. It had been predicted that a transition from granites to basalts would be observed at a depth of about 7,000 m, and that, as the bore-hole went deeper, the angle of inclination of tilted strata would gradually flatten out. But so far it has been granite all the way, though of increasing density, and the angle of inclination of the strata remains constant.

The Kola bore has still another 5,000 m to go before it reaches its scheduled depth, but already some of the preliminary data are being used in planning prospecting for new mineral deposits. The other projected bores will also have their practical aspects — the Tyumen' bore will look for deep level oil and gas deposits in Western Siberia, while the Tagil bore will investigate the "foundations" of the Urals — an important range mineralogically.

The most exciting proposal under consideration would entail drilling a bore at the foot of the Avachinskaya Sopka — a large and active volcano in Kamchatka.

Vera Rich

Uranium deposits

France backs basics

Paris

A £1 million laboratory opened last week at Nancy seems intended to give France an incontestable world lead in uranium geology. Nicknamed CREGU, the laboratory will poach its director, Dr Bernard Poty, and five senior staff from the institute next door — the Centre de Recherche Pétrographique et Géochemique, which has already established a lead in using microscopic laser Raman spectroscopy to analyse fluid inclusions in ore-bearing rocks. Such inclusions are expected also to give a clue to the formation of uranium ores — a complex process because of the extreme geochemical mobility of uranium, and the low concentrations of economically significant ores.

CREGU itself is a £400,000-a-year five-year experiment. Its £1 million buildings are a local enterprise, rented to the Commissariat à l'Energie Atomique and the five principal uranium prospecting firms in France. The Centre National de la Recherche Scientifique (which runs Poty's old lab) will pay the scientists' salaries. After five years, a council composed of members of the sponsoring organizations will decide whether the enterprise has been a success, and if it should continue.

To be successful, the new laboratory will not have to devise revolutionary methods for detecting uranium — though nobody denies this is the long-term aim of the investment. (In fact a proposal that CREGU should look at empirical methods of prospecting using magnetic anomalies was rejected by the laboratory's council.) Rather, it will attempt to extend the very sketchy existing knowledge of how the ore is formed. It is here that the fluid inclusions are significant, for they are relics of the fluids which deposited the ore — but they are so tiny that they have evaded detailed chemical analysis. CREGU, it is hoped, will solve this problem with its Raman techniques.

Other mining interests in France are watching the new laboratory carefully. It will help to train students and to retrain prospecting uranium geologists; and as ores of other minerals become economic at increasingly lower concentrations, greater and greater scientific subtlety is required in the detection of ores, and the judgement of the economic value of potential mines. So, it is believed in France, activities such as GREGU's, bridging basic science and industry, will become increasingly important.

Robert Walgate

The title 'Soviet science: relations forsworn', *Nature* 23 October, p.672, should have appeared at the top of the left-hand column of the page, to head the article by Vera Rich. The article by Robert Walgate should have been entitled 'Nuclear waste: West German problems'.

NEWS AND VIEWS

Jupiter's magnetosphere

from S.W.H. Cowley

DIRECT observations by space probes over the past decade have shown that at least three planets in our solar system, in addition to the Earth, possess an intrinsic magnetic field. These are Mercury, Jupiter and Saturn. The solar wind, a tenuous proton-electron plasma blowing radially outwards from the Sun at speeds $\sim 400 \text{ km s}^{-1}$, confines this magnetic field to a cavity surrounding the planet forming a planetary magnetosphere from which the solar wind itself is largely excluded. Only Venus is known to be without a magnetic field although limited data suggests that this may also apply to Mars.

Of the four observed magnetospheres, that surrounding the Earth is, of course, the most thoroughly investigated and the best understood. Jupiter comes second, having been the subject of four fly-by encounters, by Pioneer 10 and 11 in 1973 and 1974 and by Voyager 1 and 2 in 1979. The Pioneer flights provided the first mapping of the Jovian magnetic field and the energetic charged particle radiation trapped on Jovian field lines. The Voyagers returned the first detailed data on the thermal plasma component and on plasma waves. Here we shall describe recently published Voyager results on low-energy Jovian plasma, setting them within the context of other major Voyager findings published in *Science* (204, 1979; 206, 1979), *Nature* (280, 1979), and *Geophysical Research Letters* (7, 1980).

The properties of magnetospheric plasmas depend upon the nature of the plasma sources and sinks, and on the internal transport processes. In the Earth's magnetosphere transport is dominated by a large-scale convective flow driven by the solar wind (see Figure a). Since highly electrically-conducting plasmas and magnetic fields can be considered to be 'frozen' together we can equivalently describe the flow of field lines. Terrestrial field lines are transported by the solar wind flow from the dayside magnetosphere boundary at $\sim 10 R_E$ ($1 R_E = \text{Earth's radius} \sim 6,400 \text{ km}$) to the night side, where they are stretched out into a long $\sim 1,000 R_E$ magnetic tail. These flux tubes then flow back to the Earth through the 'centre' of the magnetosphere, heating entrained solar

wind plasma and escaped ionospheric plasma up to $\sim 10 \text{ KeV}$ energies as the tubes contract. A hot 'plasma sheet' is thus formed at the centre of the tail and a hot outer radiation zone around the Earth as shown in the Figure. Near the Earth, however, in a region usually extending to about $\sim 4 R_E$ in the equatorial plane, flows imposed by the Earth's rotation are more important than the solar wind driven convection. Here the flux tubes corotate with the Earth, their 'ends' being 'frozen' into the rotating ionosphere. This region then contains high density cold plasma from the ionosphere, and also, as a very minor constituent, energetic (Van Allen)

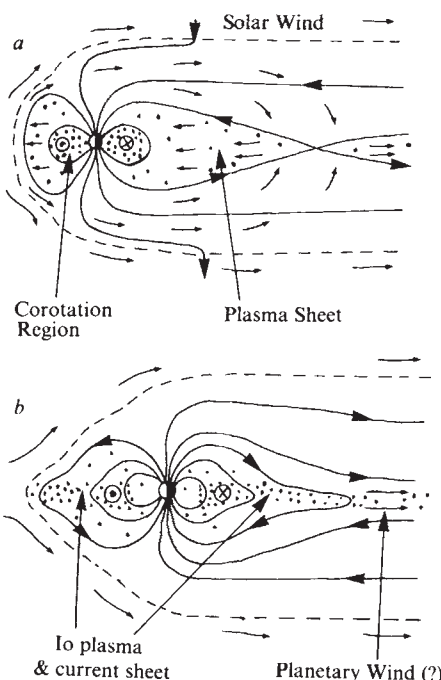
radiation belt particles. The latter are transported into the region by radial diffusion from the outer radiation zone under the action of fluctuations in the external convection, rapidly gaining energy proportional to the field strength as they move inwards.

By contrast with the Earth, flow in the main part of the Jovian magnetosphere, extending some $50 R_J$ ($1 R_J = \text{Jupiter radius} \sim 71,400 \text{ km}$) on the dayside, is entirely dominated by corotation with the planet (Figure b), although this does not preclude the existence of a long (maybe $\sim 2 \text{ AU}$) magnetic tail on the night side arising from the solar wind interaction. Thus, like the Earth's corotating 'core' of field lines, the Jovian magnetosphere is dominated by cool plasma from internal sources, but also contains radially diffusing energetic particles of solar wind origin. The radial diffusion is probably driven both by turbulent Jovian atmospheric winds at ionospheric heights and by flux tube interchange motions arising from unstable density distributions of the low energy plasma.

The second important difference between the Earth's and Jupiter's magnetosphere is that the latter is so large that it encompasses the orbits of many of the moons. These bodies can then act both as absorbers of energetic particles, as the Pioneer results demonstrated, and as important sources of low energy plasma. Indeed, the Voyager results indicate that the dominant source of thermal plasma is not Jupiter's ionosphere as might have been expected, but the satellite Io, which orbits at a radial distance of $5.9 R_J$, in the inner magnetosphere.

Voyager images show that Io is extremely volcanically active, probably due to intense internal tidal heating, thereby maintaining a tenuous atmosphere which appears to be composed mainly of sulphur dioxide. A small escape flux of this gas (small in relation to total volcanic outflow) followed by ionization then results in the formation of a dense corotating plasma torus in Io's vicinity composed mainly of sulphur and oxygen ions with energies several tens of eV. The torus has a thickness $\sim 1 R_J$, the ions being retained near the rotation equator by centrifugal forces, and a sharp inner boundary at $\sim 5 R_J$, where the temperature abruptly drops to a few eV. The latter

Sketch of (a) the terrestrial and (b) Jovian magnetospheres in noon-midnight meridian planes showing plasma flow within them. The Sun is to the left. Solid lines are field lines and the dashed line is the magnetosphere boundary. Flow in the Earth's magnetosphere is dominated by solar wind-driven convection, except near the Earth where corotation prevails and the flux tubes are filled with cold ionospheric plasma (heavy dots). In the Jovian magnetosphere corotation dominates, possibly resulting in a rotationally-driven planetary wind on the nightside. The sketches are not to scale. In linear dimension the Jovian magnetosphere is at least fifty times the size of the Earth's, and five times the size of the sun.



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observations indicate that inward radial diffusion of the ions towards Jupiter is rather slow, allowing time for the plasma to radiatively cool, thereby generating the considerable UV and optical emissions observed from the inner torus. The cooling plasma collapses towards rotation equator where the ions and electrons eventually recombine to produce relatively fast neutrals which fly off into the outer magnetosphere and solar wind.

By contrast, the outer part of the torus beyond the Io source appears to be unstable, the density gradient generating flux tube interchange motions which rapidly diffuse the plasma outwards into the magnetosphere. The plasma remains strongly concentrated at the equator, but the density rapidly drops with increasing distance due to the expanding flux tube volume as the field strength weakens. From values of several thousand cm^{-3} in the torus, the equatorial density falls to $\sim 1 \text{ cm}^{-3}$ at $\sim 25 R_J$ and to $\sim 10^{-2} \text{ cm}^{-3}$ at $100 R_J$ on the night side. In order to maintain rigid corotation, the Jovian atmosphere must continuously provide a torque on flux tubes in Io's vicinity where the plasma is created, since the corotation speed increases at a rate $\sim 12.5 \text{ km s}^{-1} R_J^{-1}$ as the particles diffuse outwards. The torque is provided by atmospheric drag of ionospheric ions at the 'feet' of the flux tubes, but theoretical estimates (Hill *J. Geophys. Res.* **84**, 6554; 1979) indicate that the coupling may be too weak to maintain rigid corotation out to large distances. Voyager low energy plasma data indicates that is so, $\sim 20\%$ reductions below expected speeds being inferred at ~ 10 to $20 R_J$. Indeed, even at $\sim 40 R_J$ the azimuthal speed is reported as remaining near 200 km s^{-1} , less than half the corotation value. However, analysis of the angular anisotropies of higher energy ions shows good agreement with rigid corotation at such distances and beyond. The reason for this disagreement is currently unknown.

The most recent results on Jovian plasma flow (Belcher & McNutt this issue of *Nature* p813) show that in addition to at least partial corotation, the low energy plasma also has comparably large flows along the field lines, directed toward the equator on the night side and away on the day side. As these authors point out, this flow most probably results from a day-night asymmetry in the magnetic field, which is compressed on the day side relative to the nightside by the dynamic pressure of the solar wind. As flux tubes rotate into the dayside sector they move closer to Jupiter and the plasma flows away from the equator due both to the field compression and to the decreasing centrifugal force. On the nightside the tubes expand and move outwards, the centrifugal force increases and the plasma collapses back toward the equator.

An important feature of a corotation-dominated magnetosphere is that if the

magnetosphere is large enough, and if corotation is maintained to large enough distances then the plasma kinetic energy density associated with the rotation will always reach and exceed the magnetic energy density at the equator and beyond a certain distance. At this point the field can no longer retain the plasma, which will break away from corotation and flow rapidly outwards forming a 'planetary wind', a rotationally-driven analogue of the solar wind. Conditions on the dayside Jovian magnetosphere, confined to $\sim 50 R_J$ by the solar wind, seem somewhat marginal for planetary wind formation and no evidence for such an outflow was seen during the Voyager inbound passes near noon. On the nightside conditions are more favourable, and an antisunward flow which may be related to planetary wind formation was observed by Voyager 2 in a layer adjacent to the dawn magnetosphere boundary $\sim 130 R_J$ down-tail from Jupiter, as shown in Figure *b*. One particularly intense burst of such flow was found to consist mainly of heavy ions streaming at $\sim 2,000 \text{ s}^{-1}$.

One final feature of Jupiter's magnetosphere which is receiving much attention is the considerable magnetic field distortions indicated in Figure *b*, due to an

equatorial sheet of current carried by magnetospheric plasma in the azimuthal direction. These field distortions are most pronounced on the nightside, where they gradually take on the character of a magnetic tail at sufficiently large distances, but they are also observed on the day side as well. To produce the effects observed beyond $\sim 10 R_J$ the equatorial plasma pressure must be comparable to the field energy density. However, the pressure of the cool (~ 10 to 100 eV) Io torus plasma appears generally to be somewhat too small to account for the observed effects, thus indicating that a significant hot component is also present. Voyager observations at energies above $\sim 30 \text{ keV}$ show that such a component is indeed present with energies ~ 20 – 30 keV and an ion composition which includes protons, oxygen and sulphur as major constituents. The heavy ions must originate at Io, but how these particles become heated to such energies remains to be determined. It therefore seems that the Jovian magnetosphere contains at least two thermal plasma components, the cool Io torus plasma which may usually dominate the mass density, and a much hotter plasma also containing Io ions which often dominates the energy density. □

Iron deprivation as a biological defence mechanism

from Thomas Emery

OVER thirty years ago Schade and Caroline discovered that blood contains a specific iron-binding protein that inhibits the growth of pathogenic bacteria, such as *Shigella dysenteriae* (*Science* **104**, 340; 1946). They correctly concluded that the protein bound iron so tightly that the bacteria were deprived of that essential nutrient and were unable to grow. When it was subsequently discovered that the protein in question, transferrin, was responsible for mammalian iron transport, the significance of the bacteriostatic action was virtually forgotten. It now appears that deprivation of invading microbes of iron may be an important defence mechanism in both animals and plants.

Microorganisms excrete low molecular weight iron chelating agents, called siderophores, when they are deprived of iron (Neill *Adv. in Chem.* **162**, 3; 1977). Siderophores are extraordinarily efficient iron chelators with binding constants of about 10^{30} , very close to the strength of transferrin. A microorganism invading the bloodstream is thus capable of

competing with transferrin for the metal. If the microbe succeeds, a potentially fatal infection may result. This battle for iron is known to occur in other animal systems where bacterial growth might otherwise be especially favourable. Milk contains lactoferrin, a protein that binds iron in a manner very similar to transferrin. Lactoferrin is also found in human secretions associated with mucosal surfaces, and there is evidence that its iron binding properties offer bacteriostatic protection (Arnold *et al. Science* **197**, 263; 1977). Another iron-binding protein, conalbumin, is found in raw egg white, and Schade and Caroline were the first to suggest that conalbumin protects eggs from infection (*Science* **100**, 14; 1944). Protection by depriving infecting microorganisms of an essential nutrient has been called 'nutritional immunity' by Weinberg (*Science* **184**, 952; 1974).

It now appears that the plant world makes use of a similar strategy. Certain bacteria, called plant growth-promoting rhizobacteria (PGPR), significantly increase yields of potato, sugar beet and other plants when they colonize the roots. Work from the laboratory of Kloepper *et al.* reported in this journal (*Nature* **286**,

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885; 1980) demonstrates that PGPR in the soil produce extracellular siderophores which strongly complex iron and make it unavailable for growth of other potentially harmful microorganisms. A new siderophore, which the authors call pseudobactin, has been isolated from PGPR. *In vitro* experiments show that pseudobactin inhibits growth of the plant pathogen, *Erwinia carotovora*, by depriving the organism of iron. The effect was also demonstrated *in vivo* by greenhouse assays in which the addition of 10 μ m pseudobactin in the water supply led to a more than a doubling of the weight of potato plants, attributed to a 74 per cent decrease in the number of pathogenic fungi found in the plant environment. Mutants of PGPR unable to produce pseudobactin did not show this effect.

A corollary to this work is that the plant itself must have a mechanism for utilizing siderophore iron lest it also be threatened by iron starvation. Virtually nothing is known about the possible importance of siderophores as a source of iron for higher plants. However, independent work reported in this journal has shown that siderophore type iron chelates are virtually ubiquitous in soils (Reid *et al.* see this issue

of *Nature*, p833). Significant siderophore production was observed in extracts of all 57 soils surveyed. Of all iron binding agents tested, only siderophores proved to be efficient biological sources of iron in soil, and they conclude that microbial siderophores may be an important source of iron for higher plants, a function previously attributed to organic acids and polyphenolic humic material.

The role of siderophores in agriculture should be a subject of serious investigation. Each year many tons of synthetic iron chelates are used to ensure adequate iron supply to plants. Natural siderophores are apparently insufficient to supply this iron, possibly because very low levels of soluble iron are known to repress the excretion of siderophores by microorganisms. An interesting approach might be to produce mutants in the laboratory that are no longer subject to this negative control. Inoculation of soils with these organisms might increase the levels of soluble iron, as siderophore chelates, to a point where no additional supplementation would be necessary. This approach would have the added advantage of suppressing the growth of plant pathogens via iron deprivation. □

The development of animal segments

from Gerold Schubiger

In this issue of *Nature* Nüsslein-Volhard and Wieschaus (see page 795) describe the isolation of mutations at 15 different gene loci that affect the pattern of segmentation in *Drosophila*. Segmentation is a phenomenon widespread in the animal kingdom; in annelids and arthropods blocks of cells become separated during development and then, within each block, the same components, such as ganglia, muscles and body cavities, differentiate. Even in the chordate embryo a metameric arrangement of some structures is obvious. Although similarity in appearance of metameric structures makes it tempting to conclude that they arise by a general developmental principle, there are still a great many unanswered questions.

We do not know if segmentation occurs by separating groups of cells from all the germ layers, or if one layer — ectoderm, neuroectoderm or mesoderm — first segments and then imprints its pattern on the other germ layers. The latter possibility is supported by observations of the leech, where the first signs of segmentation are seen in metamERICALLY arranged mesodermal founder cells, and *Drosophila*, where the first cells to reveal a segmental

pattern are the neuroblasts^{1,2}. Normally, development of the insect embryo depends on similar inductive processes, such as the induction of mesodermal differentiation by the ectoderm (see ref. 3 for a review).

The mechanism which brings about segmentation is not understood. One possibility is that positional information is used to determine segment borders. Cells of the early *Drosophila* embryo are segregated into sub-populations or polyclones assigned to develop specific segments⁴. Cells of a polyclone are restricted: that is they can form only structures of their particular segments (see, for example, ref. 5). Such clonal restrictions have been found in the abdomen of *Oncopeltus*⁶ and in the hypodermal segments of the adult *Drosophila*⁷. Restriction of clones in the hypodermis of the larva has been indicated but any clear compartmentalization of internal structure has not been documented.

It is also possible that development is primed from centres located in specific regions⁸. In some insects segmentation is first visible in the presumptive thorax region; more anterior and posterior segments boundaries arise later. In other insects, however, the role of such controlling centres is less obvious making it

very difficult to generalize.

Experimental studies indicate that the segmental pattern in *Drosophila* is already determined in the blastoderm cells⁹. Embryonic fragments isolated before the blastoderm stage form a metameric pattern which is deficient in segment number indicating that different egg parts normally interact to form the full complement of segments.

Searching for mutations and investigating their effects will tell us the extent to which segmentation is under genetic control. We already know that some genes (the homeotic) control the morphological character of segmental quality. Mutations in these genes transform patterns either within segments (for example, *engrailed*) or between different segments (for example *bithorax*)¹⁰. The mutation *bithorax* transforms the anterior metathorax into anterior mesothorax¹¹. Homozygous *bx* clones have been induced in heterozygous animals during both embryonic and larval life and always express the transformed *bithorax* phenotype, showing that the expression of the normal phenotype requires continual activity of the *bx* locus. In such homeotic mutants the number of segments is not different from wild-type so it may be concluded that these genes control segment character and not number.

Abnormal segmentation may not be a direct consequence of gene mutation. For instance, mutations such as *rudimentary*¹² block pyrimidine synthesis and produce a generally sick embryo. The autophene (enzymatic block) is responsible for many allophenic characters such as abnormal segmentation¹³. Such mutations are obviously not helpful in understanding the genetic control of segmentation. Primary defects are more likely to be detected if the earliest developmental stages are analyzed, as has been done by Nüsslein-Volhard and Wieschaus with some of the mutations. It is important to realise that some genes may not be involved in primary segmentation, but may be responsible for maintaining the proper segmentation once it has formed. Analysis of mutants should also be extended to segmentally arranged structures other than the hypodermis, since there is good evidence that in *Drosophila* inductive processes are also involved in the development of segmentally arranged tissues. This will tell us if these mutations

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interfere with induction rather than the process of segmentation.

Temperature-sensitive alleles of the most promising mutations that affect segmentation will enable us to find out when such genes are active during development. From studies of such a mutant (*patch*), Nüsslein-Volhard and Wieschaus were able to conclude that proper segmentation depends on early activity of the gene.

One mutation known to affect segmentation (*fused*) has a male-rescuable

maternal-effect¹³. Homozygous mutant offspring from heterozygous mothers can develop normally. Moreover, heterozygous embryos from homozygous mutant mothers can be rescued by the *fused*⁺ allele of the father. Thus, normal development requires the *fused* gene to be active either during oogenesis or in the zygote. This indicates that some genes that control normal segmentation are active during both oogenesis and early development. □

Cosmic spherules

from David W. Hughes

TAKE a kilogram of the sedimentary deposits from the floor of the deep oceans, wash and sieve it carefully and you will find, among a vast jumble of irregular particles, a collection of between a few hundred and a few thousand black 'stony' and 'iron' magnetic spherules with diameters between 30 and 200 μm . You don't even have to resort to deep-sea sediments; spherules can be found in arctic ice, manganese nodules, beach sands, ancient salt deposits and even on roof tops. They are a ubiquitous component of all terrestrial sediments.

The source of the spherules may be either terrestrial or cosmic, and it is the latter source that is of interest here. Collections removed from sediments laid down before the industrial revolution and its accompanying pollution make the selection process simpler but even then vulcanism is a major source of contaminants. The extraterrestrial nature of a specific spherule can be confirmed if it contains wüstite — a metastable iron oxide formed at high temperatures and low oxygen partial pressures. Wüstite slowly decomposes into α -iron and magnetite in most terrestrial environments and is thus almost unknown in nature. Having iron, nickel and cobalt in meteoritic ratios and free iron and nickel metals are also strong clues as is a low abundance of manganese, titanium and chromium.

Once a sample of cosmic spherules has been found and recognised we reach the major difficulty of deciding exactly where they come from. There are two classes of theory. The first postulates that cosmic spherules are molten spray from meteorites and meteoroids that have ablated as they decelerate in the Earth's atmosphere. The spray solidifies and drifts down to the surface. The second theory, advanced by Öpik nearly thirty years ago (*Irish Astr. J.*, 1, 145; 1951), postulates that the spherules existed essentially as spherules in free space, entered the Earth's atmosphere in such a way that they survived evaporation and then fell earthward. D. W. Parkin, R. A. L. Sullivan and J. N. Andrews of the

University of Bath, support the second theory and go even further in suggesting that cosmic spherules are condensed 'sparks' formed by collisions in the asteroidal belt. Their findings are published in a recent edition of the *Philosophical Transactions of the Royal Society of London*, 297, 495; 1980.

From the qualitative standpoint both theories seem reasonable. A quantitative assessment is more stringent. Parkin *et al.* estimate that 5,000 tonnes of spherules fall to the Earth's surface per year. The dried clay sediment contains about one part per million by mass of cosmic spherules and the mass sedimentation rate is taken as 10 g of dry clay per square metre per year. Hughes (*Space Research*, 15, 531; 1975) calculated that the total amount of interplanetary matter collected by the Earth each year is about 16,000 tonnes (upper and lower error limits being 30,000 and 8,000 tonnes respectively). So according to Parkin *et al.* spherules make up a considerable percentage of the total influx.

In 1976 Ceplecha (*Lecture Notes in Physics*, 48, 385) suggested that most of the cosmic dust in the atmosphere (and by implication in sediments) came from the disintegration of meteoroids with in-space masses of between 10^5 and 10^9 g. The influx of these objects produce brilliant fireballs. Each day there will be about 4 of – 17 magnitude, 2 of – 18 magnitude and so on in the Earth's atmosphere. The mass flux is relatively uncertain simply because considerable disagreement exists as to the luminous efficiency of the incident bodies. Also the mass influx of large bodies to the Moon, measured by Apollo seismic stations, yield much smaller values than those predicted by Ceplecha.

Obviously, particulate matter is generated by fireballs. The massive injection of dust into the atmosphere following the Tunguska event in 1908 and the great daylight fireball of 1972 illustrate

this. But what percentage of the fireball mass would end up as spherule-sized particles and what size distribution do these have? Complete ablation is common. Most fireball parents release the large majority of their mass at heights below 90 km and are still travelling at velocities above 4 km s^{-1} at the end of their luminous trails. Particles released at these low altitudes and high speeds are at least molten and in extreme cases completely vaporized. The liquid layer created at the front of the incident body by atmospheric friction will be carried round to the rear where sustained boiling may eject droplets in the vacuum wake behind the meteorite. The molten spray then cools by radiation but also undergoes considerable frictional power loading before retarding to free fall velocity. Whether this would produce a second melting or even complete evaporation would depend on the atmospheric density and particle velocity at emission. Meteorite parent bodies definitely undergo surface melting. Retrieved meteorites are covered with a millimetre or so of thick, black crust which may be either glassy (in stony meteorites) or composed of magnetic iron oxides (in iron meteorites).

Moving to the small-size end of the incident mass spectrum, it is rather remarkable how effective the Earth's atmosphere is as a collector of dust. Many incident microparticles are slowed down so gently that they remain virtually unaltered. The frictional power generated over the particle surface is low enough to be thermally radiated without the particle reaching its melting point. However, the actual temperature that the particle reaches depends strongly on its initial velocity, size and entry angle. Particles larger than 100 μm usually melt even if their initial velocities are only slightly above the escape velocity of 11.2 km s^{-1} (the minimum velocity of an incident particle). 10 μm particles can enter at 45° to the vertical with an initial velocity of 15 km s^{-1} without melting. All particles moving faster than 50 km s^{-1} burn up.

Cosmic spherules straddle this size range, the Parkin *et al.* sample having sizes between 30 and 200 μm . They would melt at a temperature of 2,000 K. All can get through the atmosphere if they have low enough velocities and, for the larger particles, if they 'graze' into the atmosphere, arriving almost tangentially.

Parkin *et al.* go even further. From scanning electron microscope observations they are convinced that none of their collected spherules melted during entry. But some incident particles must melt. The authors suggest that these do not re-solidify. At best they evaporate and at worst they are blown to pieces by the sudden release of adsorbed solar wind gases. A 100 μm lunar soil grain exposed for about 1,000 y adsorbs about 10^{-4} mol per g of ^4He , sufficient to burst a molten spherule against surface tension.

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We are left with the problem that too many spherules are found to be produced by meteorite or fireball parent body spallation alone. Indeed, according to Parkin *et al.* X-ray microprobe chemical analysis and examination with a scanning electron microscope indicate considerable differences between spherules and meteorite fusion crusts. To them the spherules seem to have survived the passage through the atmosphere relatively unscathed. Where did they come from?

The authors point to the asteroid belt and suggest that spherules are quenched sparks formed by colliding asteroids. They even went so far as to collect rounded, quenched grindwheel sparks from a wheel being used to grind a steel bar and to claim that there were distinct similarities to the deep sea spherules. Microdebris from asteroid collision would take about 10^6 y to spiral in to the Earth's orbit under the influence of the Poynting-Robertson effect, ample time to adsorb solar wind. It is even possible to

imagine a temporary hot oxidising atmosphere at the collision site due to the abundance of hydrated minerals thus producing the requisite wüstite. But are asteroid collisions frequent enough to produce 5,000 tonnes of spherules per year?

Many people think that the asteroids have been around since the dawn of the solar system and that this permanence relies to a considerable extent on a collision rate which is decreasing with time. Individual collisions may produce swarms of spherules followed by long time periods when the production rate is much smaller. The beauty of core analysis is that, assuming a constant sedimentation rate, you have a perfect means of measuring the spherule influx rate as a function of time. In a note added in proof the authors claim to be able to see evidence for individual collision events. By considering the way in which spherule size varies as a function of core depth and the spiraling time varies as a

function of particle size these events are placed in the asteroid belt.

Adherents to the first 'meteoritic ablation' theory are still not convinced. U-2 aeroplanes flying in the stratosphere at altitudes of 20 km collect many particles with meteoritic, extraterrestrial compositions. Brownlee, Blanchard, Cunningham, Beauchamp and Fruland (*J. Geophys. Res.* **80**, 4917; 1975) find that about 10 per cent of them are spherules. To them X-ray diffraction, mineralogy and texture all point to a very strong similarity between spherules and meteorite fusion crusts. It is possible that both theories are right and spherules can be formed either from ablation droplets or from quenched asteroid sparks. Indeed, still further sources are not ruled out — maybe comets contain spherules and maybe planetary cratering events produce spherules in the ejector. We have plenty of possibilities available. What is needed are more scientific investigators. □

Did the Santorini eruption destroy the Minoan world?

from Jörg Keller

THE recent report in *Nature*¹ of the discovery of volcanic ash from Santorini on the island of Rhodes raises once again the possibility that Minoan Crete was destroyed by the effects of a volcanic eruption.

Marinatos² was the first to postulate a direct connection between two of the most dramatic events in the early history of the Aegean area — the tremendously powerful eruption of the volcano of Santorini (Thera) early in the 15th century BC and the sudden and enigmatic decline of the flourishing Minoan civilization on Crete.

Seismic tidal waves, volcanic earthquakes and ash-fall were the physical effects considered most likely to have seriously damaged Minoan settlements all over Crete. A blanket of ash, thick enough to suffocate the agricultural economy on Crete, was widely thought to have fallen since deep-sea sediment cores drilled north and south of Crete had proved that the tephra had reached the island³.

Since 1967, the settlement of Akrotiri has been excavated from underneath a thick pumice cover on the island of Thera (Santorini). An unexpectedly wealthy, cosmopolitan Minoan community has come to light. Countless examples of fine pottery, both of local manufacture and imported from Crete, and frescoes of incomparable beauty were found in three-storied houses and mansions⁴. The catastrophic dimensions of the Santorini eruption are very obvious. With increasing knowledge, however, the original idea of the direct physical destruction of Crete by volcanic phenomena has become more and more questionable^{5,6}.

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Recent research in the Aegean area, both on land and in the deep sea has produced a distribution map for the Minoan ashfall^{7,8}, which shows an easterly dispersion away from the volcano towards Western Anatolia. Substantial fall-out must have affected the Eastern Aegean Sea and Western Anatolia (for example 30cm of tephra have been found on the Island of Kos⁸), but it is inferred that Crete received only a few centimeters of ash, too thin to account for the desertion of the Late Bronze age settlements.

The findings on Santorini also suggest that the volcanic destruction of the city of Akrotiri occurred one stage earlier on the pottery-based time-scale of the archaeologists than the devastation of Minoan cities and palaces on Crete. The Bronze age pottery style 'Late Minoan IA' is fully developed in Akrotiri and the pottery record ends with this stage. In contrast, all over Crete LM IA is followed by pottery of Late Minoan IB, with its highly characteristic decoration showing marine subjects. It is thought that the time difference between the two substages is about 30-50 years. This suggests a date of 1500-1480 BC for the end of Akrotiri on Santorini and 1450 BC for the sudden

end of the Minoan civilization on Crete.

Various ideas have been presented to account for the difference in the timing of the two events. Multistage volcanic activity, the possibility that the pottery might have been more old-fashioned in provincial Akrotiri, the destruction of Akrotiri by precursor earthquakes, or evacuation of the settlement some 30 years before the fatal eruption, have all been considered as explanations.

Only when Dorothy and Charles Vitaliano^{9,10} found microscopic traces of Santorini tephra in archaeological sites on Crete and Melos, and within strata believed to be uncontaminated Late Minoan IA did the possibility emerge that the climax of the Santorini eruption actually preceded the end of the Minoan civilization by about 30 years.

This interpretation seems now to be fully supported by the new findings on Rhodes. The future investigations which Doumas and Papazoglou have announced will hopefully yield a complete pottery inventory from both below and above the tephra layer. They should characterize a possible LM IB destruction level. But already their conclusions appear to be inevitable: life continued without any significant break after the ash fall. The later destruction of Minoan Crete is once more an enigma. The influence of other civilizations especially neighbouring Mycenae, will certainly have to be re-evaluated. Whether a Mycenaean invasion and conquest of Crete happened during the aftermath of one of the most catastrophic volcanic eruptions known in the Mediterranean will only be revealed by continuing research. □

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Global dendroclimatology in sight

from A. Barrie Pittock

Dendroclimatology, the systematic study of past climates using tree growth characteristics as proxy climate data, is a relatively young science. It is often confused with the much older and related science of dendrochronology, the dating of specimens by the use of tree-ring sequences. Dendroclimatology has only really developed as a science since the 1940s, primarily as a result of the pioneering efforts of workers at the Laboratory of Tree-Ring Research at the University of Arizona. Elsewhere, the serious application of dendroclimatic methods has only begun in the last five years or so.

It was, therefore, encouraging to find at a recent workshop* attended by 56 scientists from 14 countries that dendroclimatology is making great strides. Greater global coverage was reported in contributions from Europe, Asia, South America, South Africa and Australia, as well as eastern North America and the Arctic. A striking departure from early research in the semi-arid southwest United States was the documentation of useful climatic sensitivity in trees from other than obviously stressed environments. This was particularly well demonstrated for Southern Hemisphere rain forest environments by La Marche and co-workers, for the eastern United States by Cook and for Europe by several groups (Schweingruber, Eckstein, Baillie, Pilcher, and see Hughes *et al.* 272, 606; 1978; Dunwiddie & La Marche *Nature* 286, 796; 1980; Garfinkel & Brubaker *Nature* 286, 872; 1980). Major research groups (outside the western United States) now exist in several regions, including the United Kingdom, continental Europe and the eastern United States. Strong interest is also being shown in China (two workers were present at the meeting), Argentina, Australia and the Soviet Union. The Chinese plan to use tree-ring data in regions such as the northwest where historical data are not abundant.

Climatologists were also present at the conference and this led to a stimulating interaction between the potential users of dendroclimatic data and the data producers. It was suggested that attempts should be made to reconstruct a wider range of climatic variables, alongside temperature, precipitation and surface

pressure and that sampling should be concentrated in regions which are climatologically important and/or where there are gaps in coverage from instrumental, historical or other proxy data. There is growing confidence that with some modification existing methods can extract useful climatic information from trees in many different parts of the world.

As dendroclimatology has expanded into new geographical areas over the last decade, techniques have been modified and refined. One example is the use of density as well as ring-width measurements for climate reconstructions. Density data have been used recently to reconstruct temperature data in Europe (Schweingruber *et al. Tree-Ring Bulletin* 38, 61; 1978). Although basically similar, the climate-growth response appears simpler for density than ring width (Parker *Syesis* 9, 163; 1976). New techniques take into account that growth in a particular year is influenced by growth in prior years, that growth is a function of tree age and that the climate-growth response may change as trees age. A recent innovation has been the use of selective filter techniques in calculating the response and transfer functions.

Of special interest were plans for European climate reconstructions put forward by one of the global data base working groups. The plans gave high priority to building up a dense network of chronologies back to at least 1750 covering the whole of Europe. Using instrumental data from 1880 to the present for

calibration and earlier instrumental and historical data for verification, it should be possible to produce a detailed and continuous climate reconstruction from 1750. Because of the abundance of data for calibration and verification, this could prove the best continent-wide proxy climate data set in existence and Europe could become a test bed for refining dendroclimatic methods.

Five-hundred-year long chronologies are available already from living conifers in the Alps and at the northern tree-line and are expected from living oaks in Scotland, northern France and south-central Germany. These should allow the reconstructed record to extend back into the 15th century, and extension back for 1,000 years at a reduced spatial density is possible (see Serre *Tree-Ring Bulletin* 38, 25; 1978). Abundant sub-fossil and archaeological material may be used to extend the chronologies still further. However, the use of such material poses methodological problems in matching series from dead wood with living trees, especially in a way which will not distort the low-frequency climatic information. Work on these problems is being actively pursued and its importance may be gauged from the potential development of several-thousand-year chronologies from various parts of the globe including Europe, North America and New Zealand. The application of densitometric and possibly isotopic methods may well overcome some of the more serious problems in using these long tree-ring chronologies. □



100 years ago

It is stated that at the National Exhibition to be opened at Milan next year there will be a captive balloon, on the model of the one which was so successful in Paris in 1878. It will measure not less than 180 feet in circumference, 84 feet in height, and contain 15,000 cubic feet of gas. To it will be attached a safe and solid car, capable of containing seats for at least eight persons. A steam-engine is to regulate the ascent and descent, and it will rise to a height of about 900 feet, affording a splendid view of Milan and the plains of Lombardy. The balloon will be constructed at Milan, m. Henri Beudet, the well-known and experienced aeronaut, having been sent for to direct the work.

THE *Boston Herald* gives the following account of an American experiment made on September 2: — "A novel exhibition of powerful electric lights was made last evening

in the vicinity of the Sea Foam-house, Nantucket Beach, and the display was witnessed by quite a crowd of interested spectators. The Northern Electric Light Company have erected three wooden towers, each 100 feet high, and mounted upon each of these a circular row of twelve electric lights of the Weston patent, each light being estimated at 2,500-candle power. As these towers are but 500 feet apart and in a triangle, it will be seen that the light of 90,000 candles was concentrated within a limited territory. The design of the exhibition was to afford a model of the plan contemplated for lighting cities from overhead in vast areas, the estimate being that four towers to a square mile of area, each mounting lights aggregating 90,000-candle power, will suffice to flood the territory about with a light almost equal to midday. Last evening a motive power of thirty-six horses was used in generating the electricity from three Western machines, and the lights, with one single flicker, burned steadily and brilliantly all the evening. It is difficult to say whether the experiment proved anything or not. The claim put forward by the company is for an original plan of lighting cities and towns by grouping and elevating electric lights of any kind."

From *Nature* 22, 28 October, 593 & 615, 1880.

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*The Second International Workshop on Global Dendroclimatology was held at the Climatic Research Unit, University of East Anglia, July 7-11, 1980. It was sponsored by the World Meteorological Organisation, the United Nations Environment Programme, the Scientific Affairs Division of NATO and the US National Science Foundation and was organised by M. K. Hughes (Liverpool Polytechnic), P. M. Kelly (University of East Anglia) and J. P. Pilcher (Queen's University, Belfast). A report on the proceedings will be published in 1980 and a book in 1981.

REVIEW ARTICLE

Hormone families: pancreatic hormones and homologous growth factors

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The growing realization that biologically active polypeptides can be grouped in families, the members of which show structural and functional relatedness, is illustrated by the four families which are represented in the pancreas by the hormones insulin, glucagon, somatostatin and pancreatic polypeptide.

THE study of the origins of hormone-like activity is revealing great complexity in endocrine control systems, but a degree of simplicity in that most hormones belong to a family group. Members of any family are related in chemical sequence in a way which suggests their evolutionary relationships. In some cases members of a family retain common biological activities as a vestige of their common ancestry; however, for many the divergence of biological function is complete. Moreover these families are becoming extended with the inclusion of growth factors from the serum and neurotransmitters and neuroregulators from the central nervous system. These trends are well illustrated by examples drawn from the four families of peptide hormones represented in the pancreas by insulin, glucagon, somatostatin and pancreatic polypeptide.

Insulin

Porcine pancreatic insulin, shown schematically in Fig. 1, is a globular polypeptide. It has a hydrophobic core and two mainly hydrophobic surfaces which may be involved in intermolecular interactions stabilizing insulin dimers and 2-zinc insulin hexamers¹. The sequences of vertebrate insulins² (Fig. 1) show that the residues of the hydrophobic core are conserved, and that all insulins might attain the same tertiary structure as porcine insulin. Although teleost fishes, reptiles, birds and most mammals have insulins which form dimers and hexamers³, certain hystricomorph rodents such as the guinea pig and the casiragua, lack the zinc-binding histidine at position B10 (Table 1) and have lost the ability to self-associate. The insulin of the primitive cyclostome, the hagfish, also lacks B10 histidine; it can form dimers but not zinc hexamers⁴. The sequence of proinsulin^{5,6}, the precursor of insulin, is shown in Fig. 1; a C-peptide connects B30 and A1. The probable structure of proinsulin is indicated in Fig. 2. The evidence for this structure¹ is indirect and is based on the similarity of proinsulin to insulin in circular dichroism, self-association, immunological studies and the ability of the two peptides to co-crystallize.

It has been recognized for some time that two species of rodents (rat, mouse) and at least one species of fish (toadfish) contain two different insulins, almost certainly derived from two nonallelic insulin genes⁷. Goodman and co-workers⁸ have determined the sequence of the DNA for the rat insulins I and II and have shown that each contains an intervening sequence of 119 base pairs in a region corresponding to the 5' untranslated portion of the mRNA. In addition, the rat insulin II gene contains a 499 base pair intervening sequence in the region of DNA coding for the C-peptide. More recently it has been shown

that the human and chicken insulin genes resemble the rat II gene in having two intervening sequences, the second at exactly the same position in the C-peptide encoding region of the gene^{9,10}. (Could the presence of the intervening sequence in the C-peptide region account for its being the most highly variant part of the proinsulin molecule in which deletions and additions also occur?) The similarity of the human chicken and rat II genes implies that their ancestral insulin probably contained two intervening sequences. Gene sequencing also suggests that there are two non-allelic insulin genes in humans which differ by at least one base pair⁹. This would be consistent with the report of an unusual insulin, which results in diabetes and hyperproinsulinaemia and which is produced in addition to a normal insulin, implying codominant expression of two alleles¹¹.

It is not known for certain whether the two rat insulins are synthesized by the same cell. Some indication that this might be the case comes from the electron microscopy of rat insulin storage granules within a single cell which show morphologies characteristic of both insulins¹².

Insulins bind to receptors from tissues of differing species in a way which reflects the origin of the hormone rather than the receptor. Hence, with the possible exception of hystricomorphs¹³, there seems to have been evolutionary conservation of the insulin receptor. Studies of the potency and receptor binding of differing insulins and synthetic analogues have demonstrated that for full potency the general tertiary structure needs to be conserved¹⁴⁻¹⁶. Of the surface of the insulin molecule an extensive region around residues A21, B24 and B25, perhaps involving as many as eleven residues appears to be important in receptor binding.

Relaxin, a member of the insulin family?

For many years it was thought that insulin was not a member of a larger family of hormones. Recently, however, it has been suggested that relaxin, a polypeptide hormone from the corpus luteum and responsible for dilation of the symphysis pubis prior to parturition, may be a member of the insulin family. Relaxin has two chains (A and B) linked by disulphide bridges as shown in Fig. 1^{17,18}. Model building shows that the backbones of the relaxin and insulin molecules may have similar conformations (see Figs 2 and 3), although the hydrophobic core of relaxin involves different side chains, close-packed into the same volume^{19,20}. The absence of residues in relaxin equivalent to position A21 or to the B-chain C-terminal of insulin probably destabilizes the insulin fold but should not prevent its formation. The relaxin A-chain N-terminus has two extra residues and lies

A Chains		-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				
Insulin																																					
Bovine		—	—	Gly	Ile	Val	Glu	Gln	Cys	Cys	Ala	Ser	Val	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn	—	—	—	—	—	—	—	—	—				
Human		—	—	Gly	Ile	Val	Glu	Gln	Cys	Cys	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn	—	—	—	—	—	—	—	—	—				
Rat 1		—	—	Gly	Ile	Val	Asp	Gln	Cys	Cys	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn	—	—	—	—	—	—	—	—	—				
Rat 2		—	—	Gly	Ile	Val	Asp	Gln	Cys	Cys	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn	—	—	—	—	—	—	—	—	—				
Guinea pig		—	—	Gly	Ile	Val	Asp	Gln	Cys	Cys	Thr	Gly	Thr	Cys	Thr	Arg	His	Gln	Leu	Gln	Ser	Tyr	Cys	Asn	—	—	—	—	—	—	—	—	—				
Casiragua		—	—	Gly	Ile	Val	Asp	Gln	Cys	Cys	Thr	Asn	Ile	Cys	Ser	Arg	Asn	Gln	Leu	Leu	Thr	Tyr	Cys	Asn	—	—	—	—	—	—	—	—	—				
Coypu		—	—	Gly	Ile	Val	Asp	Gln	Cys	Cys	Thr	Asn	Ile	Cys	Ser	Arg	Asn	Gln	Leu	Met	Ser	Tyr	Cys	Asn	Asp*	—	—	—	—	—	—	—	—				
Hagfish		—	—	Gly	Ile	Val	Glu	Gln	Cys	Cys	His	Lys	Arg	Cys	Ser	Ile	Tyr	Asn	Leu	Gln	Asn	Tyr	Cys	Asn	—	—	—	—	—	—	—	—	—				
Insulin-like																																					
Growth factor																																					
IGF 1		—	—	Gly	Ile	Val	Asp	Glu	Cys	Cys	Phe	Arg	Ser	Cys	Asp	Leu	Arg	Arg	Leu	Glu	Met	Tyr	Cys	Ala	Pro	Leu	Lys	Pro	Ala	Lys	Ser	Ala					
IGF 2		—	—	Gly	Ile	Val	Glu	Glu	Cys	Cys	Phe	Arg	Ser	Cys	Asp	Leu	Ala	Leu	Leu	Glu	Thr	Tyr	Cys	Ala	Thr	—	—	Pro	Ala	Lys	Ser	Glu					
Relaxin																																					
Porcine		Arg	Met	Thr	Leu	Ser	Glu	Lys	Cys	Cys	Glu	Val	Gly	Cys	Ile	Arg	Lys	Asp	Ile	Ala	Arg	Leu	Cys	—	—	—	—	—	—	—	—	—	—				
B Chains		-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31			
Insulin																																					
Bovine		—	—	Phe	Val	Asn	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Ala	—			
Human		—	—	Phe	Val	Asn	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Thr	—			
Rat 1		—	—	Phe	Val	Lys	Gln	His	Leu	Cys	Gly	Pro	His	Leu	Val	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Ser	—			
Rat 2		—	—	Phe	Val	Lys	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Ser	—			
Guinea pig		—	—	Phe	Val	Ser	Arg	His	Leu	Cys	Gly	Ser	Asn	Leu	Val	Glu	Thr	Leu	Tyr	Ser	Val	Cys	Gln	Asp	Asp	Gly	Phe	Phe	Tyr	Ile	Pro	Lys	Asp	—			
Casiragua		—	—	Tyr	Val	Gly	Gln	Arg	Leu	Cys	Gly	Ser	Gln	Leu	Val	Asp	Thr	Leu	Tyr	Ser	Val	Cys	Lys	His	Arg	Gly	Phe	Tyr	Arg	Pro	Ser	Glu	—	—			
Coypu		—	—	Tyr	Val	Ser	Gln	Arg	Leu	Cys	Gly	Ser	Gln	Leu	Val	Asp	Thr	Leu	Tyr	Ser	Val	Cys	Arg	His	Arg	Gly	Phe	Tyr	Arg	Pro	Asn	Glu	—	—			
Hagfish		—	—	Arg	Thr	Thr	Gly	His	Leu	Cys	Gly	Lys	Asp	Leu	Val	Asn	Ala	Leu	Tyr	Ile	Ala	Cys	Gly	Val	Arg	Gly	Phe	Phe	Tyr	Asp	Pro	Thr	Lys	Met			
Insulin-like																																					
Growth factor																																					
IGF 1		—	—	—	Gly	Pro	Glu	Thr	Leu	Cys	Gly	Ala	Glu	Leu	Val	Asp	Ala	Leu	Gln	Phe	Val	Cys	Gly	Asp	Arg	Gly	Phe	Tyr	Phe	Asn	Lys	Pro	Thr	—			
IGF 2		Ala	Tyr	Arg	Pro	Ser	Glu	Thr	Leu	Cys	Gly	Gly	Glu	Leu	Val	Asp	Thr	Leu	Gln	Phe	Val	Cys	Gly	Asp	Arg	Gly	Phe	Tyr	Phe	Ser	Arg	Pro	Ala	—			
Relaxin																																					
Porcine		Ser	Thr	Asn	Asp	Phe	Ile	Lys	Ala	Cys	Gly	Arg	Glu	Leu	Val	Arg	Leu	Trp	Val	Glu	Ile	Cys	Gly	Val	Trp	Ser	—	—	—	—	—	—	—	—			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
Insulin																																					
Bovine		Arg	Arg	Glu	Val	Glu	Gly	Pro	Gln	Val	Glu	Ala	Leu	Glu	Leu	Ala	Gly	Gly	Pro	Gly	Ala	Gly	Gly	Leu	—	—	—	—	—	Glu	Gly	Pro	Pro	Gln	Lys	Arg	
Human		Arg	Arg	Glu	Ala	Glu	Asp	Leu	Gln	Val	Gly	Gln	Val	Glu	Leu	Gly	Gly	Gly	Pro	Gly	Ala	Gly	Ser	Leu	Gln	Pro	Leu	Ala	Leu	Glu	Gly	Ser	Leu	Gln	Lys	Arg	
Rat 1		Arg	Arg	Glu	Val	Glu	Asp	Pro	Gln	Val	Pro	Gln	Leu	Glu	Leu	Gly	Gly	Gly	Pro	Glu	Ala	Gly	Asp	Leu	Gln	Thr	Leu	Ala	Leu	Glu	Gly	Ser	Leu	Gln	Lys	Arg	
Rat 2		Arg	Arg	Glu	Val	Glu	Asp	Pro	Gln	Val	Ala	Gln	Leu	Glu	Leu	Gly	Gly	Gly	Pro	Gly	Ala	Gly	Asp	Leu	Gln	Thr	Leu	Ala	Leu	Glu	Val	Ala	Arg	Gln	Lys	Arg	
Guinea pig		X	X	Glu	Leu	Glu	Asp	Pro	Gln	Val	Glu	Gln	Thr	Glu	Leu	Gly	Met	Gly	Leu	Gly	Ala	Gly	Gly	Leu	Gln	Pro	Leu	—	—	Gln	Gln	Ala	Leu	Gln	X	X	
Insulin-like																																					
Growth factor																																					
IGF 1		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Gly	Tyr	Gly	Ser	Ser	Ser	Arg	—	—	—	—	—	—	—	—	—	Ala	Pro	Gln	Thr	—
IGF 2		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Ser	Arg	Val	Ser	Arg	Arg	Ser	Arg	—	—	—	—	—	—	—	—	—	—	—	—	

Fig. 1 The sequences of members of the insulin family of polypeptides (see ref. 2 for review). * The Asp at A22 may be incorrect; recent recalculation of amino acid composition by R. Horuk indicates one less Asp.

close to the B-chain C-terminus in the proposed structure. Preliminary sequence data from the laboratories of H. D. Niall and C. Schwabe indicate that similar structures may be assumed for rat and shark relaxins. Like insulin, relaxin is synthesized as a single chain precursor, prorelaxin. This molecule appears to be much larger than relaxin itself even though only a short connecting peptide of one or two residues would be necessary to allow the correct conformation of the hormone²¹. Although the tertiary structures of insulin and relaxin are most likely very similar, the surfaces of the two molecules are very different. It is therefore not surprising that they are not immunologically cross-reactive, and that relaxin shows neither insulin-like biological activity nor the ability to bind insulin receptors^{22,23}.

Would the similar tertiary structure of insulin and relaxin, if proven, amount to evidence of common ancestry and divergent evolution, given the low (~20%) sequence homology between the two peptides? If we begin with the assumption that there are only few stable structures for small proteins, then the folding of each structure implies restrictions on the residues if the secondary structure preference in a region is to be maintained. In particular, glycine residues alone give the unusual conformational angles that are found, for instance, at B8 and B20 of insulin, and only cystines could link the A and B chains. How significant, then, is the sequence similarity between relaxin and insulin, most of which comprises six homologous cystines and two homologous glycines? The need for a hydrophobic core further restricts the residues which can be accepted as evidence for divergent evolution. Insulin and relaxin therefore may share a similar structure, simply because it is one of comparatively few

which are globular, contain disulphide bridges, and are formed by polypeptide chains of between 50 and 80 amino acid residues; the polypeptides might have converged to this structure. Nevertheless, it remains true that no molecule lacking hormone activity has been shown to have a similar structure, a fact which may be presented as evidence in favour of divergent evolution.

Insulin-like growth factors (somatomedins)

Recently, the insulin-like growth factors (IGFs) have also been added to the insulin family. Whereas insulin and relaxin are structurally distant relatives, giving no cross-recognition at the receptor level, insulin and the IGFs are structurally and functionally more closely related. IGFs or somatomedins are polypeptides purified from human serum and from media conditioned by cultured cells. The designation somatomedin was introduced by Daughaday²⁴ for substances fulfilling the following four criteria: their concentration in serum is regulated by growth hormone, they stimulate incorporation of sulphate into proteoglycans of cartilage, they have mitogenic activity in fibroblasts and they have insulin-like effects on adipose and muscle tissue. Four factors or groups of factors meeting these criteria have been described so far: non-suppressible insulin-like activity (NSILA)²⁵; somatomedin A^{26,27}; somatomedin C²⁸; and multiplication stimulating activity (MSA)²⁹.

The concept that some or all of the four factors might be structurally related to insulin had been slowly emerging from numerous studies on their biological and chemical properties³⁰⁻³² and from the initial observation by Hintz *et al.*³³ that somatomedin-C competes with insulin for binding to the

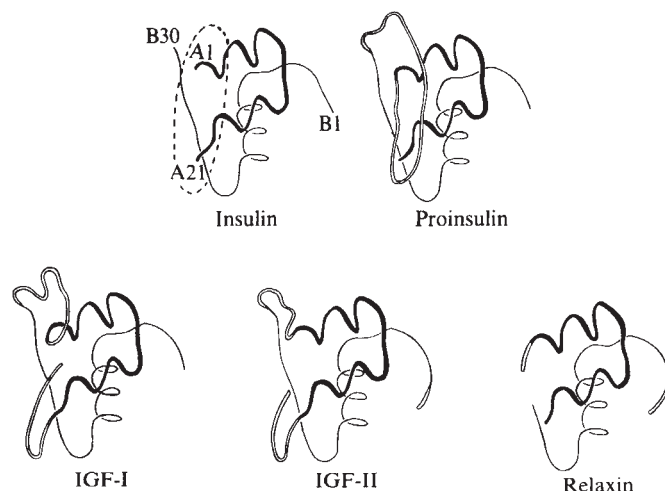


Fig. 2 Schematic diagrams showing the chain folding of insulin as derived from the X-ray analysis of insulin crystals¹ and proinsulin¹, IGF-I⁴², IGF-II and relaxin¹⁹ as proposed by model building experiments using the known sequences. The putative receptor binding region of insulin^{1,14} is indicated.

insulin receptor on cell membranes. Eventually, the isolation of two homogenous polypeptides from purified NSILA³⁴ and the subsequent determination of their amino acid sequences^{35,36} established unequivocally the long-suspected structural kinship of NSILAs to insulin and led to the new designation of insulin-like growth factors (IGFs) I and II³⁷.

A partial primary structure of somatomedin C has recently been shown to have considerable, although not complete, identity to IGF-I³⁸. However, for somatomedins A₁ and A₂, the only data so far available are amino acid compositions³⁹ which do not suggest a structural relationship to insulin and the IGFs. The same is true of several forms of rat MSA which have been purified and have had their amino acid composition determined^{40,41}. Thus out of the four groups of somatomedins, the IGFs and somatomedin C are clearly akin to insulin, whereas the putative structural relationships of somatomedin A and of the MSAs to insulin have yet to be shown.

The primary structures of IGF-I and -II in fact show more structural similarity to proinsulin than to insulin³⁵⁻³⁷ (see Fig. 1). Like proinsulin IGF-I and -II are single-chain polypeptides with three intra-chain disulphide bridges. The regions of structural similarity in IGF are confined to the B- and A-chain part of proinsulin. An intermediary or connecting region between these A- and B-chain homologous parts consists of 12 and 8 residues compared to 35 in human proinsulin with little homology between these sequences. Also IGF-I and -II exhibit a carboxy-terminal extension of 8 and 6 residues, which is not present in proinsulin.

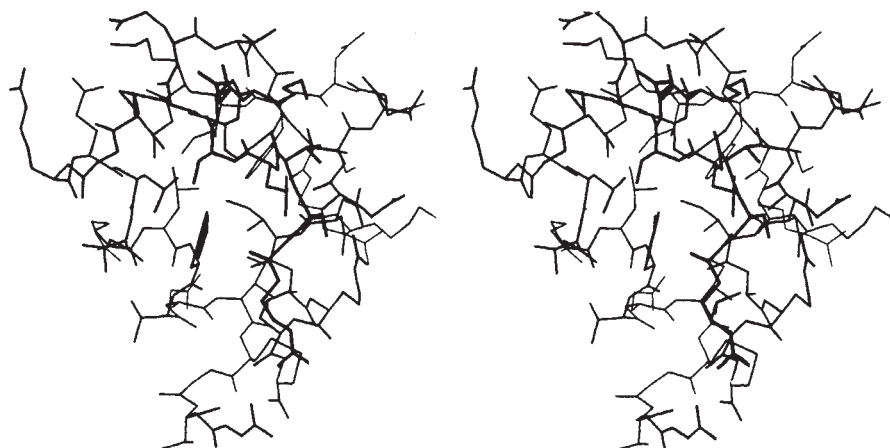
The structural similarity of IGF and proinsulin suggests a common ancestor from which these molecules diverged during evolution. From the number of identical residues between IGF and insulins from different species^{2,36}, the divergence between insulin and IGF can be surmised to have occurred before the appearance of the vertebrates provided that the rate of evolutionary change was similar in the two molecules. Likewise, the divergence between IGF-I and IGF-II can be timed at the appearance of the first mammals.

Based on the sequence similarity of IGF and insulin and the known three-dimensional structure of insulin, models have been constructed for IGF-I⁴² and IGF-II (Figs 2 and 4). These indicate that the two growth factors can have an identical hydrophobic core and an identical conformation of those residues corresponding to the A- and B-chains of insulin. Their short connecting peptides are easily accommodated in the models although they cover less of the molecular surface than the C-peptide of proinsulin; this appears to be compensated by the extension of the A-chain termini in both IGF molecules. Part of the putative receptor-binding region of insulin described above is thus modified whereas the remaining region has many residues in common.

This structural model is entirely compatible with the notion that some effects of somatomedins may be due to an interaction with the insulin receptor. Earlier studies had demonstrated that impure preparations of NSILA-s inhibit the binding of ¹²⁵I-insulin to insulin receptors in direct proportion to their insulin-like activity⁴³. However, the potency of IGF-I and -II as measured by the stimulation of the conversion of glucose to CO₂ in adipocytes is about 120 and 60 times, respectively, lower than that of insulin⁴⁴. Interestingly, Piron and de Meyts⁴⁵ could show in lymphocytes that both IGF-I and -II induce, as insulin, negative cooperativity with potencies in perfect agreement with their apparent affinity for the receptor. More recently, compelling evidence for crossreactivity of somatomedins with insulin receptors has been obtained by the use of monovalent antibodies against the insulin receptor in adipocytes. These Fab fragments derived from a patient with extreme insulin resistance produced a 30-fold rightward shift in the dose response for stimulation of glucose oxidation by both insulin and MSA⁴⁶.

The discovery that somatomedins are recognized by the insulin receptor could not explain why the somatomedins have a relatively weak insulin-like metabolic effect, while their potency in promoting growth effects in fibroblasts is about 150 times the potency of insulin⁴⁴. Since the initial studies of Morell *et al.*⁴⁷ it was therefore suspected that the metabolic effects of the somatomedins were secondary phenomena due to crossreaction with the insulin receptor, and that their main effects on growth parameters were due to an interaction with a second set of receptors of high affinity for somatomedins and a lower affinity for insulin. The studies of Van Wyk *et al.*²⁸ and Megyesi *et al.*⁴³ were the first observations of distinct somatomedin receptors.

Fig. 3 Stereo view of the three-dimensional structure proposed for relaxin based on an assumed homology with insulin. (Reproduced with permission from *Nature* 270, 449-451 (1977).)



Later, numerous other studies from several laboratories (see ref. 31) provided ample evidence for the existence of specific somatomedin receptors in several tissues. Of special interest is the observation that, relative to insulin, proinsulin is ten times more effective in binding to the MSA receptor than to the insulin receptor⁴⁸. This observation is entirely compatible with the proinsulin-like structure of IGF-I⁴². The elegant studies of King *et al.*⁴⁶ demonstrate that blockage of the insulin receptor of cultured human fibroblasts does not abolish the effect of either insulin or MSA on thymidine incorporation into DNA. It appears, therefore, that interaction with the somatomedin receptor is responsible for the growth-promoting effect of somatomedins and of insulin.

On the basis of the model-building, IGF-II with its shorter connecting peptide is expected to bind insulin receptors with a greater affinity than either IGF-I or proinsulin, a prediction borne out by studies of Zapf *et al.*⁴⁴ on adipocytes and by preliminary results of de Meyts and co-workers²² on lymphocytes. The question, however, remains whether more than one type of somatomedin receptor is present on target cells. Preliminary evidence for at least two receptors, one preferentially binding IGF-I, the other IGF-II and MSA has recently been obtained in several cell types by Rechler *et al.*⁴⁹.

In native serum, the somatomedins are bound to a large extent to at least one type of carrier protein⁵⁰ which also seems to be under the control of growth hormone^{51,52} and to be released concomitantly with somatomedin from the liver^{53,54}. The presence of such a specific and saturable binding protein in serum is unique among peptide hormones. Neither insulin⁴⁴ nor relaxin or nerve growth factor compete with the IGFs for binding. It may be speculated, therefore, that regions of the IGF molecules not homologous to insulin are making contact with the binding protein.

None of the somatomedins crossreacts with antibodies against insulin, despite the similar structures of the IGFs and of somatomedin C with insulin. However, the immunogenic region of insulin appears to be different from the receptor region and comprises residues mainly within the sequences B1-B10 and A8-A10 (refs 1, 55). The surface side chains in these regions of IGF molecules are quite different from those of any of the insulins, thus explaining the lack of cross-reactivity of the IGFs and of insulin with their respective antibodies. Antibodies against IGF-I crossreact 100-fold better with IGF-I than with IGF-II and antibodies against IGF-II 35-fold better with IGF-II than with IGF-I⁵⁶. In radioimmunoassays for IGF-I and for somatomedin C, somatomedin-C behaves almost or completely identically to IGF-I^{56,57}, whereas somatomedin A and MSA are less than 5% as potent as somatomedin-C and IGF-I⁵⁷. Surprisingly, in a radioimmunoassay for somatomedin A, IGF-I is 10 times more potent than somatomedin A⁵⁸, adding to the bewilderment about the identity of somatomedin A.

The recently developed radioimmunoassays for somatomedin C⁵⁹ and IGF-I and -II⁵⁶ have allowed the levels of these soma-

tomedins to be determined in clinical conditions such as acromegaly and hypopituitary dwarfism. IGF-I seems to be stringently regulated by the levels of circulating growth hormones whereas the levels of IGF-II are only marginally influenced by growth hormone. Thus, *in vitro*, IGF-I and -II have qualitatively identical effects with some variation in potency, whereas *in vivo* a distinct difference in regulation suggests they play different roles. Further support for different physiological functions of IGF-I and -II comes from preliminary studies showing synthesis in chick liver cells of a somatomedin similar to human IGF-I, but not to IGF-II and the presence of IGF-II, but not IGF-I, in human cerebrospinal fluid. Thus, IGF-I and the somatomedins seem to be growth-hormone-dependent growth factors, whereas a physiological role for IGF-II has yet to be found.

The last-mentioned fact adds considerably to the deplorable semantic confusion in this field. Since somatomedins are defined as growth-hormone-dependent growth factors, IGF-I and somatomedins A and C qualify as true somatomedins, whereas IGF-II does not. Conversely, somatomedins such as somatomedin A or MSA cannot unreservedly be classified as insulin-like growth factors unless and until their structures can be shown to justify such a designation.

Nerve growth factor has also been proposed as a member of the insulin family on the basis of a suggested sequence similarity (see ref. 18 for review). However, the model of NGF that can be derived from its sequence does not contain a conserved hydrophobic close-packed core and deletions give rise to a different conformation of those residues equivalent to the B-chain helix of insulin. Definition of the degree of structural homology must await the determination of the three-dimensional structure⁶⁰. It also appears that nerve growth factor is not able to compete with insulin for its receptor²³.

Glucagon

Compared to insulin, glucagon has a shorter polypeptide chain which is insufficient to allow a hydrophobic core to be shielded from the solvent. In dilute solution therefore, glucagon is flexible and many differing conformers are in equilibrium, with evidence for a stable interaction only between Val 23 and Trp 25 (ref. 61). At increasing concentrations (pH from 3.0 to 9.5) the molecules self-associate to α -helical trimers and higher oligomers⁶². An α -helical region exists between residues 6 and 27 resulting in the formation of two hydrophobic 'sticky' patches on the surface of each helix⁶³. As with insulin, these hydrophobic patches are involved in self-association; glucagon trimers are formed. A similar hydrophobic interaction may account for the formation of α -helical conformers which occur in nonpolar solvents and in the presence of phospholipids⁶⁴.

All mammalian glucagons which have been sequenced are identical² and turkey and duck glucagon show only one and two conservative changes respectively from the mammalian sequence. It therefore appears that there are strong selective

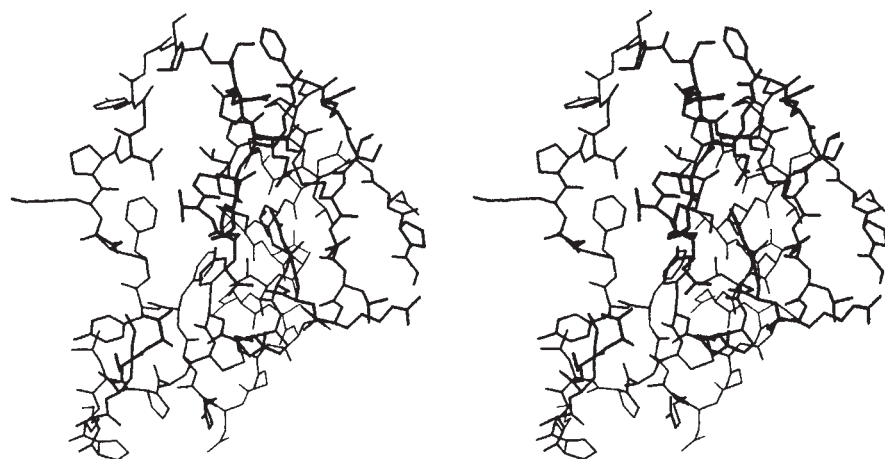


Fig. 4 Stereo view of the three-dimensional structure proposed for IGF-I based on an assumed structural homology with insulin. (Reproduced with permission from *Proc. Acad. Sci. U.S.A.* **75**, 180-184 (1978).)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
aPP	Gly	Pro	Ser	Gln	Pro	Thr	Tyr	Pro	Gly	Asp	Asp	Ala	Pro	Val	Glu	Asp	Leu	Ile
bPP	Ala	Pro	Leu	Glu	Pro	Glu	Tyr	Pro	Gly	Asp	Asn	Ala	Thr	Pro	Glu	Gln	Met	Ala
oPP	Ala	Ser	Leu	Glu	Pro	Glu	Tyr	Pro	Gly	Asp	Asn	Ala	Thr	Pro	Glu	Gln	Met	Ala
pPP	Ala	Pro	Leu	Glu	Pro	Val	Tyr	Pro	Gly	Asp	Asp	Ala	Thr	Pro	Glu	Gln	Met	Ala
cPP	Ala	Pro	Leu	Glu	Pro	Val	Tyr	Pro	Gly	Asp	Asp	Ala	Thr	Pro	Glu	Gln	Met	Ala
hPP	Ala	Pro	Leu	Glu	Pro	Val	Tyr	Pro	Gly	Asp	Asn	Ala	Thr	Pro	Glu	Gln	Met	Ala

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
aPP	Arg	Phe	Tyr	Asp	Asn	Leu	Gln	Gln	Tyr	Leu	Asn	Val	Val	Thr	Arg	His	Arg	Tyr	NH ₂
bPP	Gln	Tyr	Ala	Ala	Glu	Leu	Arg	Arg	Tyr	Ile	Asn	Met	Leu	Thr	Arg	Pro	Arg	Tyr	NH ₂
oPP	Gln	Tyr	Ala	Ala	Glu	Leu	Arg	Arg	Tyr	Ile	Asn	Met	Leu	Thr	Arg	Pro	Arg	Tyr	NH ₂
pPP	Gln	Tyr	Ala	Ala	Glu	Leu	Arg	Arg	Tyr	Ile	Asn	Met	Leu	Thr	Arg	Pro	Arg	Tyr	NH ₂
cPP	Gln	Tyr	Ala	Ala	Glu	Leu	Arg	Arg	Tyr	Ile	Asn	Met	Leu	Thr	Arg	Pro	Arg	Tyr	NH ₂
hPP	Gln	Tyr	Ala	Ala	Asp	Leu	Arg	Arg	Tyr	Ile	Asn	Met	Leu	Thr	Arg	Pro	Arg	Tyr	NH ₂

Fig. 5 The sequences of pancreatic polypeptides, PP (see ref. 23 for review). [a (avian), b (bovine), o (ovine), p (porcine), c (canine) and h (human).]

functional restraints on the glucagon molecule during evolution, although preliminary results on guinea pig, angler fish and shark glucagons indicate some divergence⁶⁵.

Study of the data on receptor binding and activity of glucagon on the glucagon-sensitive adenylate cyclase system of hepatocyte membranes led Rodbell *et al.*⁶⁶ to suggest that almost the entire molecule is required for full biological potency. Their results and subsequent detailed studies by Epand *et al.*⁶⁴ have shown that modification at the COOH-terminal or NH₂-terminal regions and on polar groups invariably leads to decreased receptor potency, although modification of some nonpolar groups (such as tyrosine by iodination) can enhance activity, indicating the importance of the whole glucagon molecule to receptor binding. Des-His¹-glucagon and other glucagons modified at the N-terminus are partial agonists with reduced affinity for the glucagon receptor suggesting that this region might be involved in hormone action whereas the major part of the molecule, including the C-terminal hydrophobic region, might be responsible for enhancing receptor affinity⁶⁷. Receptor binding studies of smaller N-terminal fragments of glucagon are consistent with this model⁶⁸. Evidence that interaction with the receptor is entropy driven suggested a model in which a helical conformer, stabilized by hydrophobic interactions, is induced or selected at the receptor from a population of conformers⁶⁵.

Glucagon is a member of a large family of homologous polypeptides, found in the alimentary tract and elsewhere, including secretin⁶⁹, the first hormone to be discovered, vasoactive intestinal polypeptide⁷⁰ (VIP) and gastric inhibitory peptide⁷¹ (GIP, now renamed glucose-dependent insulinotropic peptide) (see ref. 2 for review). Evidence for the existence of further homologous intestinal peptides⁷² indicates that the family may be much more extensive.

Glucagon is more effective than either VIP or secretin in hepatic adenylate cyclase activation and appears to act through a receptor distinct from those for secretin and VIP^{68,73}. Neither secretin nor VIP competes with ¹²⁵I-glucagon at the receptor on hepatocytes. In view of the close homology at the N-terminal region of glucagon, VIP and secretin, these observations might indicate that the C-terminal residues of glucagon are responsible for selecting the correct receptor. However, recent results indicate that the N-terminal region may also play a role in receptor selection since glucagon₁₋₆ is a partial agonist of glucagon, whereas the equivalent regions of secretin and VIP are not⁷⁴. Secretin and VIP are both recognized by each other's receptors although each binds to its own receptor with greater affinity^{75,76}. The results of these and many other comparative studies indicate that the secretin and VIP receptors, although perhaps not the glucagon receptor, have evolved from a common ancestor. Confirmation of this proposal must await chemical characterization of the receptors themselves.

Glucagon also has an ill defined relationship to glicentin, a 100-amino acid polypeptide isolated from the intestine. Glicentin is known to carry the immunodeterminants of glucagon and to account for most of the glucagon-like activity in the intestine⁷⁷. Although it is tempting to believe that glicentin is the precursor of glucagon, particularly since it is found in the specific

glucagon-containing granules of the pancreatic α -cell, recent evidence suggests that glicentin and glucagon are in topographically separated granules⁷⁸.

Pancreatic polypeptide and somatostatin

Two further peptides which may be classified as hormones are produced by specific cells of the islets of Langerhans. Pancreatic polypeptide^{79,80} is a 36-amino acid peptide which appears to have gastrointestinal functions and may act as a satiety factor; it is released after a protein meal and leads to a decrease in weight of certain genetically diabetic mice^{81,82}. Since Tatemoko and Mutt⁷² find a homologous peptide in pig intestine, pancreatic polypeptide may be a member of a larger family of gastroenteric pancreatic hormones. All sequenced pancreatic polypeptides have amidated carboxytermini and there is close homology amongst the mammalian sequences^{79,80} (Fig. 5). In contrast to glucagon, however, there are considerable differences between mammalian and avian pancreatic polypeptide sequences^{79,80}. This is also reflected in the species specificity of immunological and some biological activities⁸⁰⁻⁸².

Although this polypeptide is only seven residues larger than glucagon, it appears to have a relatively stable tertiary structure⁸³⁻⁸⁵ comprising a polyproline II-like chain segment folded against an α -helical region as shown in Fig. 6. In a similar way to insulin the molecules can self-associate to dimers. It seems that all mammalian and avian pancreatic polypeptides can attain this conformation and can dimerize; in addition the avian polypeptide, like insulin, can form higher oligomers in the presence of zinc.

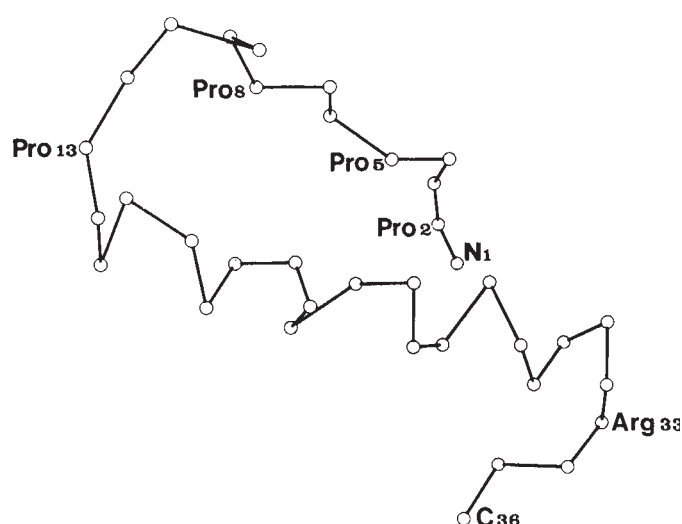


Fig. 6 The conformation of avian pancreatic polypeptide, PP, as determined by X-ray analysis. The α -helix and the polyproline-like helix pack together to give a compact structure. A similar structure may also be assumed by bovine pancreatic polypeptide, even though they are species specific in some of their biological activities. (Unpublished results of J. Pitts, S. P. Wood, I. J. Tickle and T. L. Blundell.)

Somatostatin⁸⁶ is the smallest of the pancreatic hormones. It contains 14 amino acids with one disulphide bridge; in fact an even smaller, relatively rigid peptide of residues 7–10 can be a potent agonist⁸⁷. Somatostatin is widely distributed and was originally identified in the hypothalamus, although peptides in different organs and in different mammals (pig and sheep) seem to be identical. In general the peptide appears to act locally—in the pancreas it inhibits the release of insulin and glucagon; it is therefore strictly a local hormone or paracrine agent.

Evolution of pancreatic hormones and relation to gastroenteric and neuroendocrine systems

Finally, we consider whether pancreatic hormones or close relatives are also active in the nervous system. This question is being approached by the study of the developmental and evolutionary origin of hormone-producing cells and by attempts to localise and even extract the hormones from the central nervous system (see ref. 88 for a review).

Examples of pancreatic hormone-like activity are found in the alimentary tract (see ref. 89 for review) of both protostomian and deuterostomian lines of invertebrate evolution. For instance, an insulin-like substance which has a role in carbohydrate metabolism is found in the mucosa of the alimentary tract of molluscs⁹⁰. Evidence for this insulin-like activity is derived from biological and radioimmunological assay, as well as ultrastructural, histological and histochemical techniques, but it has not yet been chemically characterized.

Van Noorden and Falkmer⁸⁹ have traced the movement of the four pancreatic hormones from the alimentary canal into the islets during vertebrate evolution. They have shown that the first hormone to be found in a well defined islet is insulin in cyclostomes such as the hagfish. Somatostatin also appears in the islet at that stage. Glucagon is the third hormone to reach the pancreas, being restricted to the gut mucosa in cyclostomes, but appearing in the pancreatic islets of the holocephalan cartilaginous fishes. In these fish pancreatic polypeptide immunoactivity is still located in the gut mucosa, but at the next evolutionary stage, the elasmobranchian cartilaginous fish, this molecule finally moves into the pancreatic islets.

Immunoreactive evidence of all pancreatic hormones has been observed in the central nervous system. Somatostatin is found immunocytochemically in the neurones of the N-paraventricularis of the hypothalamus and in other hypothalamic nuclei⁹¹. Insulin-like activity in the blowfly⁹² has its origins in the median neurosecretory cells, and in the hookworm⁹³ an insulin-like molecule of the corpus cardiacum/corpus allatum capable of decreasing haemolymph trehalose, may also derive from neurosecretory cells. Much interest has been stimulated by the recent reports of Roth *et al.*^{94,95} that insulin-like protein levels in many rat tissues including brain extracts are higher than in plasma. The marked similarity between this insulin-like activity and pancreatic insulin in radioimmunoassay, radio-receptor assay, and bioassay indicates that all tissues contain either genuine insulin or a very similar polypeptide rather than a proinsulin-like peptide or insulin-like growth factor. Roth *et al.*⁹⁴ find that genetically obese (*ob/ob*) diabetic mice in which circulating insulin is markedly increased, and insulin receptors in liver, fat and other tissues are severely depressed, have normal concentrations of insulin and insulin receptors in the brain. These and other related observations on rats made hypoinsulinaemic and diabetic by treatment with streptozotocin have been held to indicate the synthesis of insulin locally in the brain and other extrapancreatic tissues, and to suggest a possible role of insulin in neurotransmission, either as a neurotransmitter itself or as a neuromodulator. However, these reports have been disputed by Eng and Yalow who contend that insulin concentrations in the brain of larger animals never exceed plasma levels⁹⁶. Much work remains to establish whether or not insulin is synthesized in the mammalian central nervous system; the high levels of insulin may well result from

insulin which has crossed the blood-brain barrier, diffused through the brain and concentrated on brain receptor sites⁹⁶. The discovery of many of the gastroenteric pancreatic hormones in the central nervous system of higher vertebrates indicates that neuronal peptide production may represent a primitive capability. The explanation of this distribution of peptide hormones may lie in the APUD theory of Pearse^{88,97} which proposes that the embryonic neuroectoderm of nerves and peptide hormone-producing cells have a common origin in the neuroendocrine-programmed cells of the ectoblast. The diffuse regulatory mechanism of the neuronal system may have become partially replaced by aggregation of cells of the endocrine system. These ideas do not preclude an even more primitive evolution of hormones and neurotransmitters from lysosomal proteases as suggested by Hales⁹⁸. At the level of molecular evolution, duplication of ancestral genes—perhaps coding for neurotransmitters—must have given rise through natural selection of mutated sequences to a series of hormones and growth factors: families of molecules with homologous sequences.

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ARTICLES

Thermal history of the H-group of chondritic meteorites

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The Tieschitz unequilibrated H-group chondrite accreted at $(800 \pm 100)^\circ\text{C}$ during rapid cooling¹¹. From their mineral chemistry, nine other H-group chondrites, exhibiting greater degrees of equilibration, also formed hot. Degree of equilibration is equated with slowness of cooling, especially below about 700°C . If metamorphism is defined as change brought about by an increase in temperature, in the H-group chondrites it is recognized only in the localized effects of transient reheating, probably induced by shock.

THE ordinary chondrites are stony types composed of silicate, metal and sulphide phases. Because of this mixture and because they were never completely melted, the ordinary chondrites are considered to represent primitive, condensed matter of the early Solar System. Chemically they are subdivided into three groups. One of these, the high iron (H-) group, has a total iron content >24.5 wt% and iron-nickel metal generally in the range 12–21 wt%. Many members of this group have been unaffected by shock events which post-date crystallization and which caused veining, 'blackening' or brecciation in most members of the other two chemical groups^{1–3}. The H-group, therefore, seemed most appropriate to re-investigate using modern techniques. Our work will be published in detail elsewhere^{4,5}. Here, in summarizing our data, we favour a simpler early history for the H-group parent body (or parent bodies) than is currently accepted. Furthermore, we interpret for the first time the cooling history of all petrological types consistently in terms of the chemistry of both silicates and metal.

On a textural and mineralogical basis, Van Schmus and Wood⁶ subdivided the chondrites into six petrological types. Types 1 and 2 do not occur in the ordinary chondrites. Type 3 refers to unequilibrated chondrites with prominent chondrules, sub-spherical masses of silicate, some of which are quenched liquid droplets, the remainder were fashioned from solid or plastic pre-existing rock^{7,8}. In contrast, petrological type 6 applies to meteorites in which chondrules have crystallized and intergrown with the matrix. Types 4 and 5 exhibit integration of chondrules with matrices intermediate between that in types 3 and 6. The pyroxenes and feldspars of type 4 ordinary chondrites are partly chemically heterogeneous, but almost all of those in types 5 and 6 are not. In petrological type 3, metal and sulphide may form rounded droplets—metallic chondrules^{9,10}—outside silicate chondrules, or they may occur separately or together as droplets within silicate chondrules. But in higher petrological types metal and sulphide are generally absent from silicate chondrules and occur as grains with irregular outlines

indicative of partial to complete crystallization to fill the available spaces between the chondrules.

For our study, 10 H-group chondrites (named in Fig. 2) were chosen from the collection in the British Museum (Natural History) as unshocked or lightly shocked representatives of the different petrological types. The wavelength-dispersive electron microprobe at the British Museum (Natural History) was used for analysis of metal and sulphide; other phases were analysed using the energy-dispersive microprobe system at Cambridge. The 1-MV transmission electron microscope at Birmingham University provided information on the fine-structure of the chondrites.

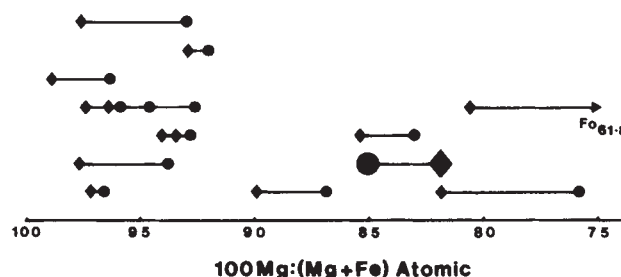


Fig. 1 Compositions of the 'cores' of co-existing phenocrysts of olivine (●) and low-calcium pyroxene (◆) in 12 Tieschitz chondrules. Except in one case (large symbols), pyroxenes are more magnesian than olivines. Because the plane of the section cuts each crystal at random, we cannot be sure that an analysis is representative of a crystal core; this accounts for the compositional differences shown. The vertical axis has no significance.

Silicate mineralogy

Tieschitz is the only highly unequilibrated chondrite among the 10 studied. In both olivines and low-calcium pyroxenes the molecular ratio of 100 Mg: (Mg + Fe) is variable within crystals and between crystals. In every zoned crystal studied the core is more magnesian than the rim, consistent with an earlier observation on the Sharps, H3 chondrite⁷; pyroxene cores are usually poorer in calcium than the rims¹¹. Of some 40 chondrules, 12 contain phenocrysts of both olivine and low-calcium pyroxene. Of these, 11 have pyroxenes more magnesian than the co-existing olivine (Fig. 1). In Tieschitz the bias in favour of pyroxene in the partitioning of magnesium between pyroxene and olivine was undoubtedly the result of crystallization of chondrule liquids. In our sample of Bremervörde (H3/4) and in the chondrites of higher petrological type, on average, the 100 Mg: (Mg + Fe) ratio is ~2% higher in low-calcium pyroxenes than in the co-existing olivines; this is consistent with earlier data¹². There is thus little difference in the partitioning of Mg between pyroxene and olivine among chondrites which exhibit different degrees of equilibration.

In Tieschitz and Bremervörde, low-calcium pyroxenes are typically of the polysynthetically twinned, monoclinic variety; they may have crystallized directly from chondrule liquids during rapid cooling but they are more probably inverted protopyroxene^{5,13}. Most pyroxene cores contain <0.75 wt% calcium (Fig. 2), as do those in the remaining, more equilibrated chondrites. Although our calcium determinations broadly agree with those quoted by Dodd¹², we observe no correlation between calcium content of pyroxene cores and the increasing petrological type. Calcium-rich pyroxene occurs in both Tieschitz and Bremervörde as overgrowths of pigeonite on twinned, low-calcium pyroxene cores or as the minor phase in intergrowths with low-calcium, monoclinic pyroxene. Pigeonites exhibit exsolution on ~10 nm scale. Diopside has not been observed, but some chondrule mesostases are rich in normative diopside. In the two least equilibrated chondrites, crystallization of olivine

or low-calcium clinopyroxene or protopyroxene resulted in the enrichment in calcium of residual chondrule liquids. Cooling was so rapid, however, that only pigeonite was able to crystallize on a pre-existing substrate of low-calcium pyroxene⁵. In the petrological type 4 and 5 chondrites the low-calcium pyroxene is typically the striated orthopyroxene which has been interpreted as 'the disordered product of inversion from a high-temperature polymorph'¹³. In the H4 chondrites, low-calcium pyroxene cores may have overgrowths of pigeonite or even subcalcic augite; augite and diopside, however, were encountered only as components (normative) in chondrule mesostases, except in the TEM study of Quenggouk⁵. Here, some chondrule mesostases contain tiny crystallites of orthopyroxene, diopside, and plagioclase. The diopside lacks exsolution lamellae, even on the finest, sub-micrometre scale. It is evidence of slower cooling and a closer approach to equilibrium crystallization than by the pigeonites in Tieschitz and Bremervörde. In the two chondrites of petrological type 5, orthopyroxene or striated pyroxene often has overgrowths of augite or diopside which are resolvable by microprobe. Finally, in the H6 chondrites the low-calcium pyroxenes are represented by orthorhombic crystals only, and diopside occurs both as overgrowths and as individual, anhedral grains.

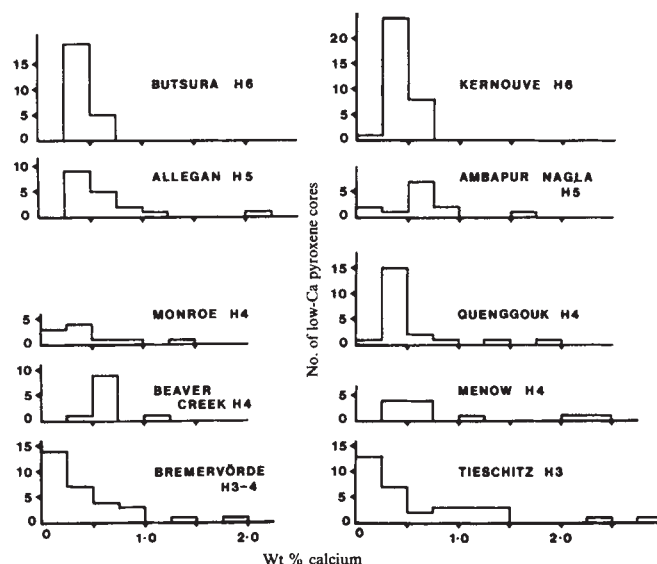


Fig. 2 Calcium content of low-calcium pyroxene 'cores' in 10 H-group chondrites representative of the range in petrological types. It may be significant that higher modal calcium contents occur in the pyroxenes of Ambapur Nagla and Beaver Creek, both of which suffered reheating.

The sequence from Tieschitz (H3) to Butsura and Kernouve (both H6) is one of increasing equilibration. In Tieschitz especially, but also in Bremervörde and the chondrites of type 4, olivines and monoclinic, low-calcium pyroxenes undoubtedly crystallized from chondrule liquids. Structurally, the 'striated orthopyroxene' of Allegan (H5) is intermediate between these monoclinic, low-calcium pyroxenes and the orthorhombic pyroxenes of Butsura and Kernouve. It is surprising that the low-calcium pyroxenes have uniformly low calcium contents throughout (Fig. 2). In addition, the partition of magnesium between olivines and pyroxenes always favours the latter. Had crystal-liquid and solid-solid equilibria been responsible for elemental partition between the phases at the respective extremes of the petrological sequence, systematic compositional differences should be evident. In their absence we conclude that, throughout the sequence, elemental partition was established by

a single process acting in uniform physical conditions. In types 3 and 4, this process was undoubtedly crystallization from chondrule liquids; this must also be true for the type 6 chondrites which do contain relict chondrules. It seems, therefore, that in the H6 chondrites the partition of calcium between the pyroxenes was dominated by the crystallization of large amounts of very low-calcium clinopyroxene or protopyroxene from chondrule liquids. Subsequently, inversion to the orthorhombic form occurred. Had orthorhombic pyroxenes crystallized directly from liquids, the crystals should contain a higher calcium content or exsolution lamellae of calcium-rich pyroxene, as for example, do the bronzites of Bushveld type¹⁴. On the contrary, even on a sub-micrometre scale, no exsolution lamellae of calcium-rich pyroxene were found in the bronzites of Butsura or Kernouve.

Conventional mineral thermometers^{15,16} are based on equilibrium partition of an element between two co-existing solid phases; a metamorphic environment has to be assumed. Our evidence indicates that these thermometers may not apply to the H-group chondrites, and this might also be true for the oxygen isotopic thermometer¹⁷. However, in addition to the above arguments based on the mineral chemistry of olivines and pyroxenes, we have observed chemical heterogeneity among the feldspars and chromites of Butsura. Thus, in at least one H6 chondrite, equilibration was not completely achieved; this conclusion was reached by Bunch and Olsen¹⁵, who used data on pairs of crystals in contact for their assessment of the various mineralogical thermometers.

Metallography

It has been argued¹⁰ that in Tieschitz, metallic chondrules and droplets in silicate chondrules cooled rapidly enough to preserve structural and chemical evidence of an origin by fractional crystallization from Fe-Ni-S melts. This is based on an interpretation of polycrystalline taenite as the relict of a solidification structure (Fig. 3a). In Tieschitz this does not seem to be in doubt because there is ample evidence of quenching in silicate chondrules and the meteorite is completely unshocked^{5,11,18}. However, polycrystalline taenite occurs also in Bremervörde, Menow, Quenggouk, Butsura (Fig. 3b) and, rarely, even in Kernouve, the most equilibrated of our sample of H-group chondrites. Is then the polycrystalline taenite in H6 chondrites a relict solidification structure, or could it have formed in some other way? If the former is true, then the H6 chondrites could not have been metamorphosed during a reheating episode at a temperature above 700 °C, for this would have caused all metal to homogenize as the γ phase. Subsequent, slow cooling could not produce the crystal boundaries in the parent γ . Internal grain boundaries in γ crystals, if produced by deformation at high temperature, could be stabilized at low temperature only by quenching or by the widespread precipitation of α Fe-Ni along the crystal boundaries. Crystallites of α are very rarely observed in polycrystalline taenite. Finally, there is no evidence that polycrystalline taenite could have formed by brittle deformation of taenite below 700 °C.

We are forced to conclude that polycrystalline taenite in H6 chondrites is a relict solidification structure. For its preservation in Tieschitz, Bevan and Axon¹⁰ state that cooling to below about 700 °C (entry of metal into the $\alpha + \gamma$ field) took days to weeks. In Butsura and Kernouve, polycrystalline taenite is rarer and more modified than in Tieschitz, indicating slower cooling in the case of the H6 chondrites; they may have taken weeks to months to cool from over 1,000 °C to below about 700 °C. However, the most important conclusion from these observations is that compositional variation in the metal of H-group chondrites was established at temperatures above 700 °C, and subsequently enhanced by the effects of solid-state diffusion at lower temperatures.

Discussion

Wood⁹ modelled the development of nickel-distribution profiles in metal grains in the ordinary chondrites. He began, however,

by assuming that above ~700 °C all metal was in the form of homogeneous γ crystals. On cooling into the ($\alpha + \gamma$) field, α nucleated and grew at the expense of γ by solid-state diffusion of nickel. By computer modelling the build-up of nickel relative to grain-size in taenite (γ), Wood estimated the rates at which various ordinary chondrites cooled through about 500 °C. The presence of polycrystalline taenite in some of the H-group chondrites indicates that above 700 °C, in the γ field, metal was inhomogeneous and that initial nickel gradients had already been established by crystal-liquid fractionation. Therefore, Wood's model⁹ must always underestimate the rate of cooling in the H-group chondrites, although for the type 6 meteorites it will produce a closer approximation to the true cooling rate than for the H3, Tieschitz.

Interpretation of polycrystalline taenite as a relict solidification structure parallels our conclusion that the

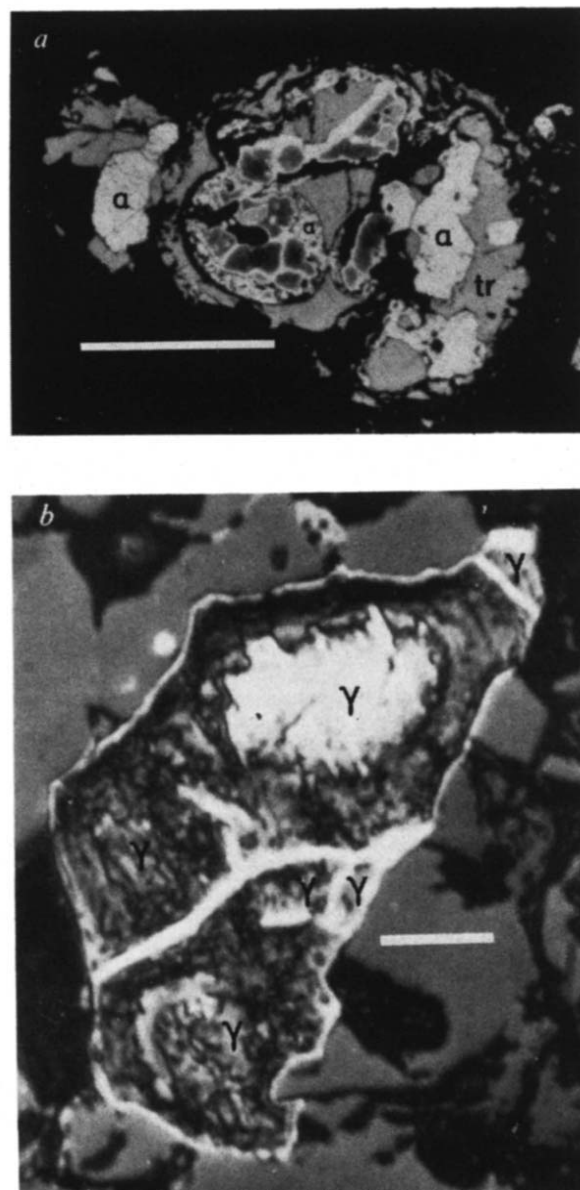


Fig. 3 a, Metal-sulphide chondrule in Tieschitz (H3). Scale bar, 100 μ m. tr, troilite; α , kamacite; γ , taenite. Two adjacent crystallites of polycrystalline taenite (γ) are indicated; each has a dark-etching core and a rim of clear taenite. b, Polycrystalline taenite, Butsura (H6). Scale bar, 20 μ m. Clear taenite (white) bounds the grain and wholly or partly delimits six crystallites (γ) each with a martensitic, dark-etching centre.

distribution of magnesium between olivine and low-calcium pyroxene, and of calcium between low-calcium and calcium-rich pyroxenes, was established by crystal-liquid equilibria during the crystallization of chondrule liquids. Moreover, survival of polycrystalline taenite and/or of monoclinic or 'striated' low-calcium pyroxene in H5 and H6 (refs 13, 19) chondrites indicates fairly rapid cooling to about 700 °C, after which there could have been no prolonged reheating at higher temperatures. 'Metamorphism' in the strict sense apparently then played no part in determining the major textural features in the H-group chondrites. Rather, we favour a model of hot accretion during cooling at various rates; H3 chondrites were rapidly cooled

low-calcium pyroxenes remove Dodd's main objections to a self-annealing ('autometamorphic') model for the thermal history of the H-group chondrites.

In Fig. 4 the generally accepted, progressive metamorphic model is compared with our preferred self-annealing model. The latter is favoured by the synchronous retention of plutonium-fission xenon, within 10 Myr, by H4, H5 and H6 chondrites; also in support are the revised plutonium-fission cooling rates²¹ of H4, H5 and H6 chondrites. Furthermore, ³⁹Ar-⁴⁰Ar ages of H-group chondrites indicate that representatives of all petrological types began to retain radiogenic argon within a 60-Myr period from 4.51 to 4.45 Gyr ago, all ages being within experimental error of the mean²². Such synchronism seems less readily attainable if the history had been complex (that is, accretion at 200 °C followed by heating to metamorphic temperatures then slow cooling to gas retention temperature) rather than simple.

Support for the metamorphic hypothesis must now be based on the interpretation of the abundances of the volatile metals thallium, bismuth and indium. It has been argued that these elements were introduced into chondritic parent bodies as part of a low-temperature fraction which became mixed with a more abundant high-temperature fraction. Calculations based on the condensation of a gas of solar composition fix the temperature of accretion within a few tens of degrees of 200 °C (Fig. 4a) (ref. 23). However, the oxygen isotopic ratios favour 'condensation and accretion at high temperature'²⁴, and we have already argued that Tieschitz aggregated at higher temperature¹¹ and that an ambient gas was probably depleted in hydrogen relative to the solar composition. Uranium-lead systematics indicate that, at room temperature, chondrites become contaminated with lead from the terrestrial atmosphere²⁵. We argue, therefore, that the H-group chondrites could have received their complement of volatile metals during cooling on a small, planetary body from ~700 °C, over 10⁵-10⁷ yr.

For either model of the thermal history our conclusions point unambiguously to a stratified parent body of the H-group chondrites. Slowly cooled, petrological type 6 material derives from greater depth than the near-surface location of type 3 which may have been within 20 m of the planetary surface¹⁰. As a corollary, this parent body could not have been externally heated after accretion of the H-group chondrite material.

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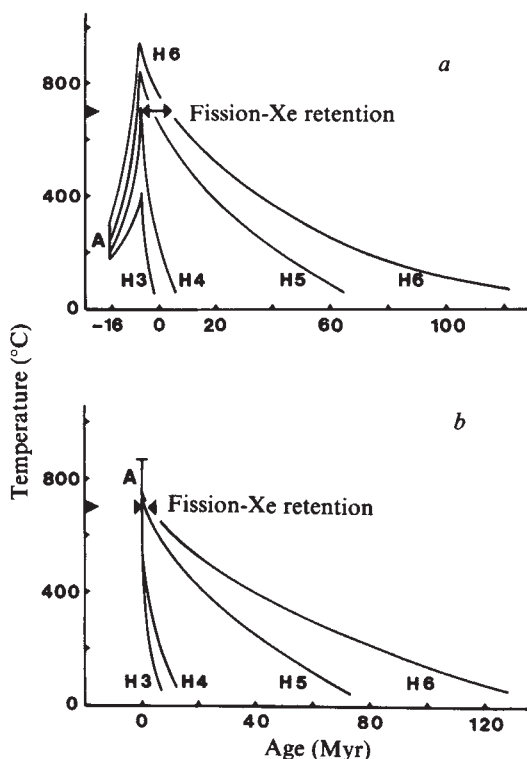


Fig. 4 Two schematic models of the cooling-history of H-group chondrites: *a*, metamorphic; *b*, self-annealing. 'A' indicates the approximate temperature of accretion. 'Fission-Xe retention' indicates the temperature at which half of the Pu-fission Xe is retained by phosphate during cooling; this temperature was attained by H4, H5 and H6 chondrites within 10 Myr (ref. 21). In both models zero time is set within this 10-Myr span. In the metamorphic model *a*, cold accretion may have taken place up to about 16 Myr before the final cooling through 700 °C. The time-limit is fixed by the content of iodine-correlated ¹²⁹Xe, which is released from specimens in the laboratory between 1,100 and 1,500 °C (refs 26, 27). Model *b*, inferred from the mineralogy, seems preferable because of its simplicity.

whereas H6 meteorites cooled more slowly, which allowed crystallization and some solid-state diffusion to occur by a self-annealing process. Based on textural and mineralogical observations, Fredriksson (see ref. 20) has argued against metamorphism in ordinary chondrites. However, the meteorites chosen are brecciated and, we believe, from Fredriksson's evidence, metamorphism followed by further chondrule formation cannot be ruled out. Dodd¹² favoured 'progressive metamorphism' as the process responsible for the varying degrees of equilibration exhibited by the ordinary chondrites. Our new interpretation of the cooling history of the metal and our observations on the uniformity of the calcium contents of the

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Measuring metamorphic history of unequilibrated ordinary chondrites

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A thermoluminescence sensitivity technique is used to give a new measurement of the degree of metamorphism of unequilibrated ordinary chondrites. Consequently the petrological assignment of these meteorites is modified.

THE wide range of textures in chondritic meteorites indicates a variety of metamorphic histories¹⁻⁴. Most meteorites are metamorphosed and it is estimated that they have experienced maximum temperatures of around 1,200 °C (ref. 5). A few (described as type 3 in the scheme of Van Schmus and Wood⁶) have apparently experienced little or no metamorphic heating and their relatively unaltered state makes them interesting subjects for study⁷. We report here measurements which seem to enable the degree of metamorphism experienced by these unequilibrated ordinary chondrites to be quantified. Based on our findings, we propose a modification to the petrological type assignment of these meteorites.

We have used a thermoluminescence (TL) sensitivity technique, sensitivity being defined as the amount of TL induced in a sample by a standard radiation dose. Any previously accumulated TL is first drained by annealing the sample to ≥ 500 °C. The TL sensitivity, or TL per rad of absorbed radiation, is an intrinsic property of the luminescent mineral in the meteorite. It should not be confused with the level of natural TL, which is additionally governed by a complex interplay between the thermal and radiation environment of the meteorite.

This work was prompted by a study of H chondrites organized by Hutchison²⁹. H. chondrites are distinguished from the other ordinary chondrites (L and LL) on the basis of bulk chemistry, but the TL properties of the three ordinary chondrite classes are remarkably similar. This is presumably because TL is produced almost entirely by feldspar¹², which is present in the same proportions and has similar composition in each group⁸. When the results of our H-group study were available, it was decided to examine some type 3 meteorites. Most of the observed falls studied by Dodd *et al.*⁹ were included (Table 1). Our measurements were made at the University of Leicester, UK¹⁰ and at Washington University, St Louis¹¹. Samples were lightly crushed and the metal and adhering sulphide removed with a hand-magnet. Three samples produced brown powders due to small quantities of terrestrial corrosion (Kernouvé, Butsura and Limerick). These were shaken for a few seconds in 10 M HCl to restore their normal grey colour, thus reducing an albedo effect which reduces TL¹². The nonmagnetic portions of the chondrites were ground fine enough to pass through a 100- μ m sieve and then heated to 500 °C to remove their natural TL. Leicester samples were given a test dose of 50 krad from a ⁶⁰Co γ source, whereas the St Louis samples were given a test dose of 25 krad with a ⁹⁰Sr β source. Samples of the Dhajala meteorite were run on both sets of apparatus and used for normalization to enable a comparison of data from the two laboratories. Using different test doses introduces a small error, but this is not thought to be

significant in view of the large effects discovered. The TL sensitivity measurements are reproducible to within $\pm 5\%$.

Results

Three plots of TL sensitivity against sample temperature ('glow curves') are shown in Fig. 1. These three curves demonstrate the range of shapes observed, although most meteorites produce glow curves which closely resemble those from Khohar. Variations in glow-curve shape are caused by the presence of several separate TL peaks whose relative contributions vary slightly. The shape variations seem unrelated to any of the factors considered here. We have also looked at the best method of extracting data from the curves; two possibilities are to measure the peak height or the area under the curve. We found no difference in the two approaches and present here the former. It also makes very little difference if the TL is measured at a given temperature rather than at the peak, as the glow curves are generally so similar and the effects discovered so large.

The thermoluminescence data are shown in Fig. 2 and listed in Table 1. The average TL sensitivity of the highly metamorphosed type 5 and 6 meteorites is appreciably higher than for the type 3 samples. Furthermore, the TL sensitivities range over a factor of ≤ 10 in samples of type 4-6 compared with a range of $\sim 1,000$ for type 3 chondrites.

A detailed examination of the accuracy of the TL sensitivity measurements for type 4-6 meteorites has been given else-

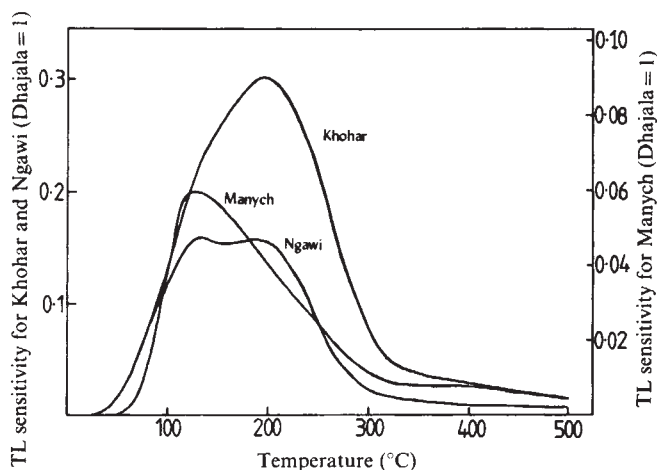


Fig. 1 Plots of thermoluminescence (TL) sensitivity—the TL induced in a sample by a standard laboratory test dose—against temperature to which the sample has been heated ('glow curves'). The TL is emitted mainly in a broad peak between 100 and 300 °C. Most chondrites studied here have glow-curve shapes resembling Khohar, but a few, like Manych and Ngawi, have shapes which differ in detail.

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where¹³. The standard deviation in 10 different meteorites which were expected to have similar TL sensitivity was 21%. Type 3 meteorites are highly inhomogeneous and only small quantities were available for our measurements, so a second chip taken from a different location on the available sample was run. The root-mean-square deviation on the duplicate values is 48%. The error bars on Figs 3 and 4 are $\pm 1 \sigma$ of the means.

Discussion

Several parameters for assessing the extent of equilibration in type 3 meteorites have been discussed, but none of them covers the range observed for TL sensitivity or is as free from ambiguity as TL (Fig. 3). The best indication of unequilibration available to Van Schmus and Wood⁶ was the inhomogeneity of the silicates. This is expressed as the per cent mean deviation (PMD) shown by the fayalite content of the olivine or pyroxene. The olivine data of Dodd *et al.*⁹ are compared with the TL data in Fig. 3a; the pyroxene data give a similar plot, but the range in pyroxene PMD is smaller. The inverse correlation indicates that

as the meteorites equilibrate, TL sensitivity increases and the silicates become more homogeneous.

In an analogous way, the range of Co contents of the matrix kamacite becomes smaller as the meteorite becomes more equilibrated⁷; we have calculated the PMD of Co in the kamacite and added our own unpublished Tieschitz datum (Fig. 3b). Again, there is a clear negative relationship. Figure 3c compares our data with the percentage matrix recrystallization¹⁴. There is some uncertainty in this measurement because it is necessary to discriminate between chondrule fragments and other non-matrix components, but there is a trend in which TL sensitivity increases with the percentage of recrystallized matrix. Huss *et al.*¹⁴ have shown that the FeO content of olivine in the matrix (normalized to the same parameter for the whole rock) decreases with equilibration. One possibility is that FeO is reduced out of the matrix and into the metal, consistent with the views of Wood¹⁵ and Afiattalab and Wasson⁷. This parameter also shows an inverse correlation with TL sensitivity (Fig. 3d).

Changes in bulk chemistry can be related to metamorphism in ordinary chondrites, although the causes of these relationships

Table 1 Samples used in the present study, their TL sensitivity, and the laboratory at which the measurements were made

	Class†	Source and catalogue no.‡	Sample weight (g)	TL sensitivity (Dhajala = 1)		Lab. §
				Replicates	Mean	
Kernouvé	H6	BM 43400	~0.100		25	L
Barwell	L5	BM 1965, 57	~0.100		16	L
Ambapur Nagla	H5	BM 81117	1.57		15	L
Appley Bridge	LL6	BM 1920, 40	3.86		14	L
Allegan	H5	BM 1920, 281	~0.100		8.6	L
Ogi	H6	BM 55256	~0.100		8.3	L
Jilin (Kirin)	H5	CAS	~0.100		7.7	L
Butsura	H6	BM 34795	0.69		7.5	L
Limerick	H5		~0.100		6.0	L
Quenggouk	H4	BM 33764	~0.100		6.0	L
Mangwendi	LL6	BM 1934, 839	0.450		6.0	L
Olivenza	LL5	BM 1925, 430	0.870		6.0	L
Beaver Creek	H4	BM 73646	0.995		2.6	L
Bremervörde	H3	BM 33910	0.880		2.6	L
Saratov	L4	BM 1956	~0.100		2.5	L
Monroe	H4	BM 25462	0.796		1.8	L
Dhajala	H3	PRL (T67)	0.30	1.0	1.0	L/SL
Mező-Madaras	(L)3	HNM	0.030	1.3	0.9	SL
			0.037	0.96		
			0.027	0.82		
			0.031	0.51		
			0.038	0.85		
Hedjaz	(L)3	MHNP 2132	0.042	0.78	0.82	SL
			0.032	0.30		
Khohar	L3	ASU 623.1	0.039	0.58	0.44	SL
			0.034	0.57		
Parnallee	(LL)3	BM 34792	0.034	0.26	0.42	SL
			0.029	0.16		
Ngawi	(LL)3	USNM 2483(4)	0.025	0.34	0.25	SL
Tieschitz	H3/L*	BM 1975, M11		0.092		L
		NHMc 2140	0.038	0.20	0.23	SL
			0.039	0.23		
			0.034	0.27		
			0.035	0.22		
Chainpur	(LL)3	ASU 424.1(4)	0.032	0.061	0.078	SL
			0.058	0.078		
Sharps	H3	NHNM 640	0.039	0.044	0.071	SL
			0.032	0.098		
Manych	(L)3	AS 2331	0.032	0.061	0.070	SL
			0.058	0.078		
Bishunpur	(L)3/H*	ASU 618.8	0.030	0.0073	0.0054	SL
				0.0034		
Semarkona	(LL)3/H*	USNM 1805	0.032	0.0061	0.0045	SL
			0.036	0.0029		
Krymka	(L)3	AS 1707	0.032	0.0035	0.0027	SL
			0.053	0.0019		

† Ref. 6. The chemical classifications of type 3 chondrites are often uncertain. Those marked with an asterisk are based on Co content of kamacite (ref. 7, except our own unpublished value for Tieschitz).

‡ BM, Dr R. Hutchison, British Museum (Natural History), London; PRL, Professor D. Lal, Physical Research Laboratory, Ahmedabad, Navrangpura, India; CAS, Drs Ouyang Ziyang and Lin Shunsheng, Chinese Academy of Sciences, Guiyang, China; AS, Academy of Sciences, USSR (through J. T. Wasson); ASU, Arizona State University, Tempe (through J. T. Wasson); HNM, Hungarian National Museum (through J. T. Wasson); NHM, Naturhistorisches Museum, Vienna (through J. T. Wasson); MHNP, Musée Histoire Naturelle, Paris (through J. T. Wasson); USNM and NHNM, Dr K. Fredriksson or Dr R. S. Clarke, Smithsonian Institution, Washington (through J. T. Wasson).

§ L, Leicester; SL, St Louis.

|| Samples were powders taken from a larger reservoir of homogenized, nonmagnetic powder of unknown sample weight.

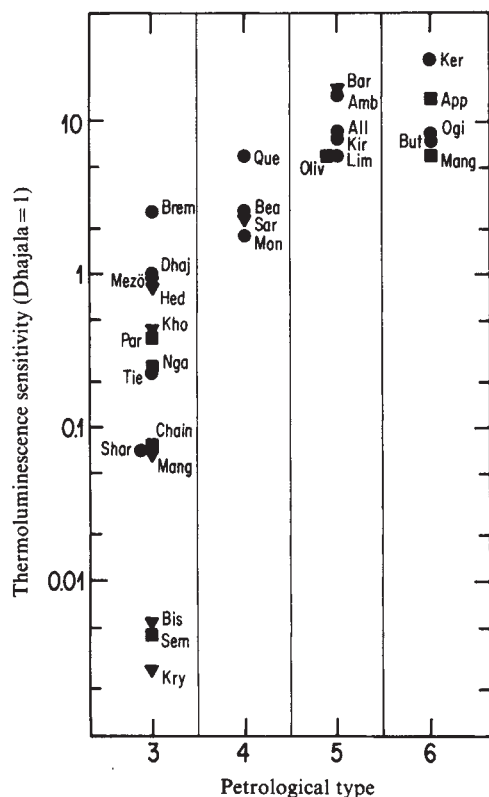


Fig. 2 Plot of TL sensitivity against petrological type for 29 meteorites, normalized to Dhajala. Note the much greater range displayed by type 3 compared with the other types. In this and Fig. 3, meteorites are identified by the first three letters of their names (see Table 1) or some simple variant. ■, LL chondrites; ▼, L chondrites; ●, H chondrites.

are debatable^{4,16-18}. The carbon⁶ and the primordial ³⁶Ar (ref. 19) contents of chondrites decrease with petrological type. These are compared with TL sensitivity in Fig. 4 (the sources of the ³⁶Ar and C data are given in the legend). These, too, display clear inverse relationships with TL sensitivity.

A relationship between TL sensitivity and petrological type was first suggested by Liener and Geiss²⁰, but their data fail to display such a relationship because only two of their 70 specimens are unequilibrated¹². The relationship between TL sensitivity and petrological type is believed to be associated with

changes in the crystalline structure of the phosphor, which in most ordinary chondrites is feldspar²¹. However, in unequilibrated ordinary chondrites, the feldspathic material is in the form of a glass⁶. Glasses are intrinsically poorer phosphors than crystalline substances of the same composition because the TL mechanism involves lattice defects or impurity ions in a crystal lattice¹⁶. The small TL sensitivity that glasses possess is probably due to localized areas of short-ranged order. The transition from glass to crystalline feldspar takes place in meteorites of type 3. In type 4, glass is generally absent, having been replaced by microcrystalline feldspar, although there may be occasional instances of turbid glass. In meteorites of type 5, glass is entirely absent and in type 6, the feldspar has grown to clear, coarse grains⁶. It seems reasonable, therefore, that TL sensitivity should be rather sensitive to the extent of equilibration in type 3 meteorites.

It has been proposed^{22,23} that radiation damage will also cause changes in TL sensitivity. Evidence for this has long been sought^{20,22,23}, but the largest effects so far observed²⁴ are at most of the order of 25%. In fact, there is some evidence¹² that they may be zero for the TL emitted between 100 and 300 °C (Fig. 1).

The reheating and shock believed to have been suffered by the black chondrites causes a decrease in TL sensitivity comparable with the range observed here. The cause is similar to that responsible for the relationship between TL and petrological type; shock causes a vitrification of the feldspar²⁵. The shock events required to affect TL are very large (≥ 250 kbar) and also cause loss of ⁴⁰Ar—reflected in K–Ar ages very much less than 4,600 Myr—and characteristic changes in the metallography. None of these meteorites are among those examined for shock effects by Heymann²⁴, although Wood¹⁵ describes Manyash as reheated and Huss *et al.*¹⁴ mention shock effects in their descriptions of type 3 chondrites. Of the three meteorites with the lowest TL sensitivity in the present suite of specimens, ⁴⁰Ar data are available for two of them. Assuming a K content of 800 p.p.m., taking ⁴⁰Ar contents from ref. 26 and using the usual decay constants and other data, these meteorites have K–Ar ages of 4.060 Myr (Bishunpur) and 4,100 Myr (Krymka), where in each case the uncertainty is probably $\pm 15\%$. It seems unlikely that these meteorites have had their TL appreciably lowered by a recent shock event of the magnitude responsible for the low TL sensitivities which have been observed in L chondrites¹². However, we cannot rule out some complex relationship between very early shock and petrological type which also implicated TL.

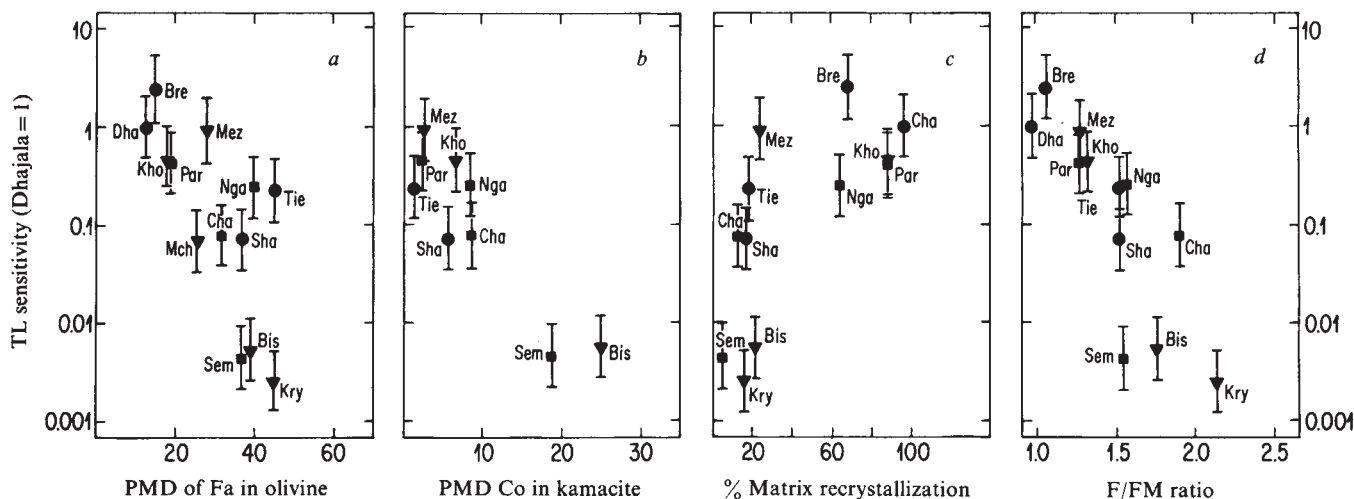


Fig. 3 Plots comparing the TL sensitivity of the type 3 meteorites studied here with literature data which attempt to measure the extent of metamorphic equilibration. *a*, Per cent mean deviation of the FeO content of the olivine⁹. *b*, Per cent mean deviation of the Co content of the kamacite (calculated from data in ref. 7 with our own unpublished data for Tieschitz). These two parameters decrease during metamorphism as the silicate and metal compositions become more homogeneous. *c*, Per cent matrix recrystallization¹⁴. *d*, FeO/(FeO + MgO) in the matrix normalized to the same parameter for whole-rock¹⁴. This last parameter decreases with equilibration as FeO is apparently reduced out of the matrix. ■, LL chondrites; ▼, L chondrites; ●, H chondrites.

Table 2 Subdivision of petrological type 3

Type	Member*	TL sensitivity (Dhajala = 1)	PMD olivine†	PMD metal‡	Matrix recrystal- lization (%)§	F/FM ratio	C w/w%¶	³⁶ Ar (10 ⁻⁸ cm ³ STP g ⁻¹) #
3.9	<i>Clovis no. 1</i> <i>Prairie Dog Creek</i> <i>Carraweena</i> <i>Bremervörde</i>	2.2–4.6	5–15	<1.5	>60	<1.0	≤0.21	<4
3.8	Dhajala	1.0–2.2	15–20	1.5–2.5	>60	1.0–1.1	0.21–0.24	4–8
3.7	Mezö–Madaras Hedjaz	0.46–1.0	20–25	2.5–4.0	>60	1.1–1.2	0.24–0.27	8–13
3.6	Khojar Parnallee Ngawi Tieschitz	0.22–0.46	25–29	4.0–6.0	>60	1.2–1.3	0.27–0.30	13–18
3.5		0.10–0.22	29–33	6.0–8.0	~50	1.3–1.4	0.30–0.33	18–27
3.4	Chainpur Sharps Maynch	0.046–0.10	33–36	8.0–10	~20	1.4–1.5	0.33–0.38	27–35
3.3		0.22–0.046	36–38	10–13	10–20	1.5–1.6	0.38–0.43	35–45
3.2		0.010–0.022	38–40	13–17	10–20	1.6–1.7	0.43–0.50	45–55
3.1	Bishunpur	0.0046–0.0010	40–42	17–21	10–20	1.7–1.9	0.50–0.60	55–65
3.0	Semarkona Krymka	<0.0046	>42	>21	<10	>1.9	≥0.60	≥65

* Meteorites in italics were not examined by TL and their present assignment is tentative being based entirely on literature data.

† % mean deviation of fayalite in olivine⁹.

‡ % mean deviation of Co in kamacite⁷.

§ Ref. 14.

|| FeO/(FeO + MgO) in the matrix normalized to the same quantity for the whole-rock¹⁴.

¶ Ref. 27.

Ref. 26.

Subdivision of petrological type 3

In many respects, and especially in their TL properties, the unequilibrated ordinary chondrites display a more excessive behaviour than shown by any other petrological type. For instance, Bishunpur bears as little similarity to Dhajala, another type 3, as Dhajala does to Kernouvé, a type 6 chondrite. To emphasize this point, we believe that some subdivision of petrological type 3 is desirable. There is no doubt that the metamorphism experienced by type 3 chondrites is gradational and shows no hiatuses. This is reflected in most of the data in Figs 2–4. (The only exception seems to be a possible discontinuity in matrix recrystallization.) This is also true of the more equilibrated meteorites. The sharpness of chondrule outlines and an olivine PMD of 5 are examples of parameters used to define higher petrological type boundaries (5–6 and 3–4 respectively), but neither is a sharp, single-temperature effect and both reflect gradational processes. The lack of hiatuses in the equilibration parameters for type 3 chondrites, such as TL sensitivity, is not an argument against subdivision.

Afiattalab and Wasson have already proposed subdivision on the basis of metal homogeneity; they divided their nine unequilibrated ordinary chondrites into: four with 'high'; two with 'moderate'; and three with 'low' metal uniformity⁷. Huss *et al.* have proposed subdivision into four categories which they described with Roman numerals¹⁴. The utility of any subdivision scheme is governed not only by its necessity but also by its simplicity and consistency with existing schemes. Ideally, any subdivision should merge into existing schemes so naturally that it is largely self-explanatory. Terms like 'highly', 'moderate' and 'somewhat' unequilibrated are cumbersome; they cannot always be neatly incorporated into graphs or tables. Letter abbreviations, such as L3h, L3m and L31, are confusing because current practice is to use letters for chemical class and numbers for petrological types. The simplest approach is to modify the present system by using decimal numbers. We propose that type 3 be considered to consist of 10 subdivisions going from type 3.0 to type 3.9. It might be logical to go from 2.5 to 3.4, so that the numbers round off to 3, but we believe our proposed range will be more popular.

Table 2 summarizes our proposed subdivision scheme for type 3 ordinary chondrites, based on the trends observed in Figs 3 and 4. Three of the subdivisions are unpopulated at present, but we see no obvious reason why these will not eventually be filled.

One subdivision contains members which we have not examined, but literature data (mainly PMD olivine) suggest that they should be assigned to petrological type 3.9. They were included for the sake of completeness because they are more equilibrated type 3 members than those examined here. It is possible to assign a petrological subtype on the basis of each of the parameters in Table 2, and such assignments are generally in very good agreement with the TL sensitivity assignment. (We have arbitrarily chosen the upper value where data fall on the boundary between subtypes.) In general, most petrological subtype assignments are within ±0.1 of those listed in Table 2, and this is the uncertainty we would assign to the petrological

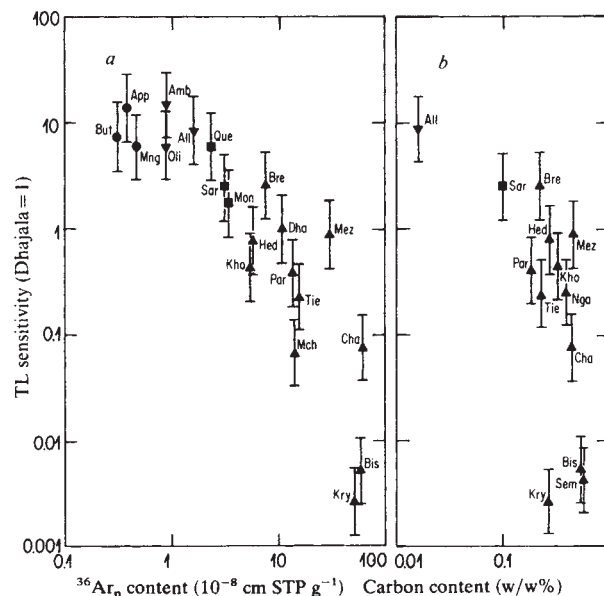


Fig. 4 Two bulk chemical parameters which are known to decrease with increasing equilibration during metamorphism, compared with TL sensitivity; a, primordial ³⁶Ar content, calculated from data in ref. 26 and unpublished supplements, assuming primordial ³⁶Ar/³⁸Ar = 5.6 and spallogenic ³⁶Ar/³⁸Ar = 0.65 (ref. 28); and b, carbon content. As with TL, the range of primordial ³⁶Ar and C contents is much greater in meteorites of type 3 than any other type. Types: ▲, 3; ■, 4; ▼, 5; ●, 6.

subtype of a given meteorite. There are only three meteorites for which the literature data assignments differ systematically from the TL assignment; the Mezö-Madaras subtype could be decreased by 0.1, the Parnallee value increased by 0.1 and the Semarkona value increased by 0.2. Clearly there would be no problem in assigning a petrological subtype in the absence of TL data, provided there were other good quality data available.

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Mutations affecting segment number and polarity in *Drosophila*

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In systematic searches for embryonic lethal mutants of Drosophila melanogaster we have identified 15 loci which when mutated alter the segmental pattern of the larva. These loci probably represent the majority of such genes in Drosophila. The phenotypes of the mutant embryos indicate that the process of segmentation involves at least three levels of spatial organization: the entire egg as developmental unit, a repeat unit with the length of two segments, and the individual segment.

THE construction of complex form from similar repeating units is a basic feature of spatial organisation in all higher animals. Very little is known for any organism about the genes involved in this process. In *Drosophila*, the metameric nature of the pattern is most obvious in the thoracic and abdominal segments of the larval epidermis and we are attempting to identify all loci required for the establishment of this pattern. The identification of these genes and the description of their phenotypes should lead to a better understanding of the general mechanisms responsible for the formation of metameric patterns.

In *Drosophila*, the anlagen for the individual segments arise as equally sized subdivisions of the blastoderm, each segment represented by a transverse strip of about three or four cell diameters¹. A cell lineage restriction between neighbouring segments is established at or soon after this stage². Two basic types of mutation have been described which change the segmental pattern of the *Drosophila* larva. Maternal effect mutations like *bicaudal* lead to a global alteration of the embryonic pattern³. Bicaudal embryos develop two posterior ends arranged in mirror-image symmetry, and lack head, thorax and anterior abdomen. The *bicaudal* phenotype suggests that the initial spatial organisation of the egg established during oogenesis involves a morphogen gradient that defines antero-posterior coordinates in early embryonic pattern formation^{3,4}. The subdivision of the embryo into segments is thought to occur by a differential response of the zygotic genome to the maternal gradient. Homeotic mutations (for example, *bithorax*^{5,6}) seem to be involved in a final step of this response process. These mutations change the identity of individual segments; for example, *Ultrabithorax* transforms the metathoracic and first

abdominal segments into mesothoracic segments. However, the homeotic loci do not affect the total number, size or polarity of the segments, nor do they point to any other step which might intervene between the maternal gradient and the final pattern of segments.

We have undertaken a systematic search for mutations that affect the segmental pattern depending on the zygotic genome. We describe here mutations at 15 loci which show one of three novel types of pattern alteration: pattern duplication in each segment (segment polarity mutants; six loci), pattern deletion in alternating segments (pair-rule mutants; six loci) and deletion of a group of adjacent segments (gap mutants; three loci) (Table 1, Fig. 1).

The segmental pattern of the normal *Drosophila* larva

Figure 2 shows the cuticular pattern of a normal *Drosophila* larva shortly after hatching. The larval body is comprised of three thoracic and eight abdominal segments. Although differences are observed in different body regions, all segments have certain morphological features in common. The anterior of each segment is marked with a band of denticles, most of which point posteriorly. The posterior part of each segment is naked. The segment borders run along the anterior margins of the denticle bands⁷, they have no special morphological features. The polarity of the pattern is indicated by the orientation of the denticle bands and, in the abdomen, by the shape of the bands (Fig. 3). In the thoracic segments the bands are narrow with fine denticles whereas those in the abdominal segments are broader and comprised of thick pigmented denticles (for a detailed description of the cuticular pattern see ref. 1).

Segment polarity mutants: deletions in each segment

Mutants in this class have the normal number of segments. However, in each segment a defined fraction of the normal pattern is deleted and the remainder is present as a mirror-image duplication. The duplicated part is posterior to the 'normal' part and has reversed polarity (Figs 1–3).

Six such loci have been identified. Three loci, *fused*⁸, *wingless* (ref. 9 and G. Struhl, personal communication) and *cubitus interruptus*^D, were previously known whereas *gooseberry*, *hedgehog* and *patch* are new (Table 1). All the mutations in this class are zygotic lethals and the phenotypes are only produced in homozygous embryos. One of the loci, *fused*, also shows a maternal effect in that a wild-type allele in the mother is sufficient to rescue the mutant phenotype of the homozygous embryos.

In all mutants except *patch* the region deleted includes the naked posterior part of the pattern and the duplication involves a substantial fraction of the anterior denticle band. In mutant larvae, the ventral side of each segment is almost entirely covered with denticles, the posterior fraction of which point anteriorly. The segment identity seems to be normal, the denticles of the abdominal segments being large and pigmented whereas those of the thorax are short and pale (Figs 1, 2). The anterior margin of the region duplicated in these mutants coincides with the segment boundary in only two cases, *fused* and *gooseberry* (Fig. 3). In *wingless* and *hedgehog* it lies posterior to

the boundary, such that these larvae apparently lack all segment boundaries.

The phenotype of embryos homozygous for *patch* contrasts with that produced by the five loci described above in that the duplicated region includes some naked cuticle anterior to each denticle band. The duplicated unit thus involves structures of two adjacent segments. *Patch* larvae, despite the normal number of denticle bands, have twice the normal number of segment boundaries (Figs 1, 3).

Despite these differences, the common feature of all mutants in this class is that a defined fraction of the pattern in each segment is deleted. This deletion is associated with a mirror image duplication of the remaining part of the pattern. We suggest that these loci are involved in the specification of the basic pattern of the segmental units.

Pair-rule mutants: deletions in alternating segments

In mutants of this class homologous parts of the pattern are deleted in every other segment. Each of the six loci is characterized by its own specific pattern of deletions (Table 1, Figs 1, 4). For example, in *even-skipped* larvae, the denticle bands and adjacent naked cuticle of the pro- and metathoracic, and the 2nd, 4th, 6th and 8th abdominal segments are lacking. This results in larvae with half the normal number of denticle bands separated by enlarged regions of naked cuticle (Figs 1, 4). In *paired* larvae the apparent reduction in segment number results

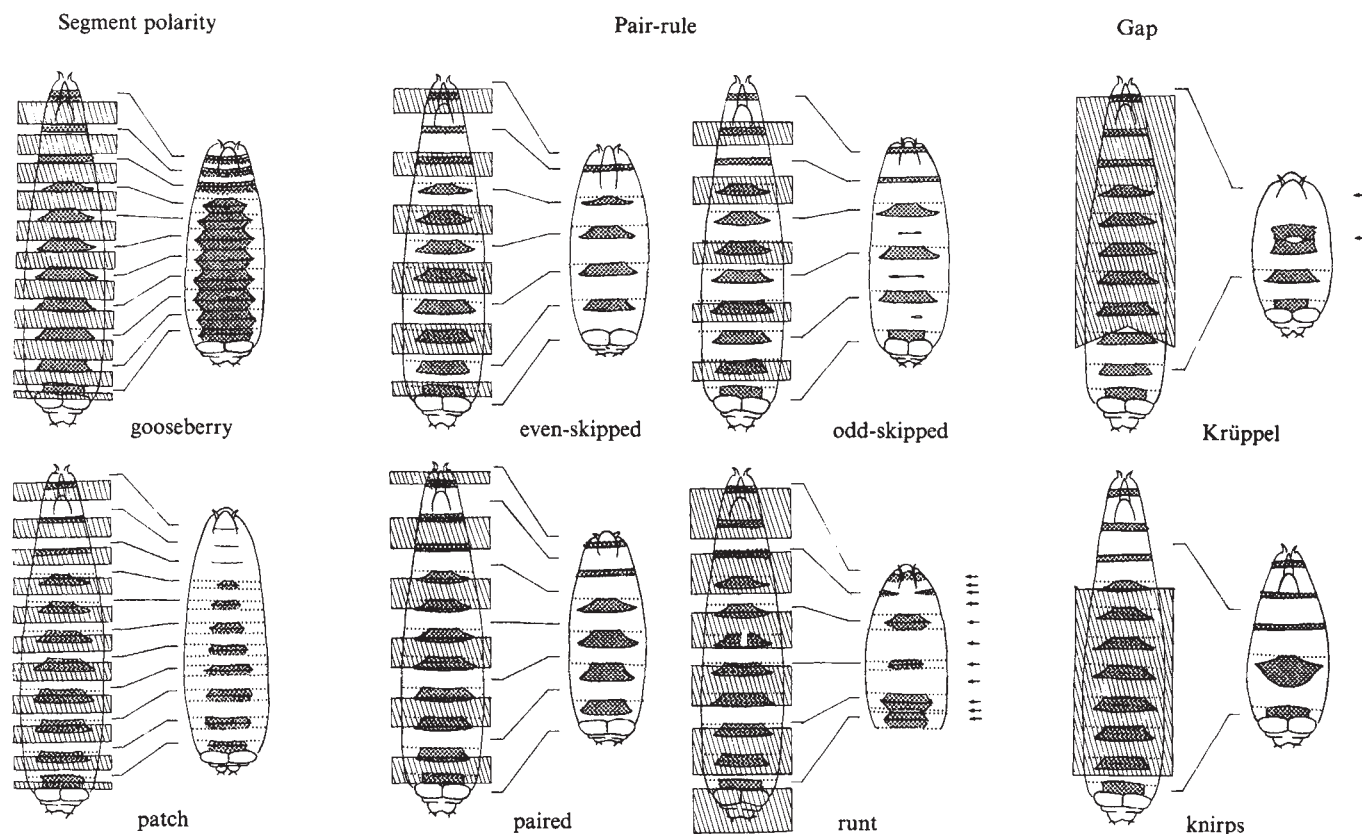


Fig. 1 Semi-schematic drawings indicating the regions deleted from the normal pattern in mutant larvae. Dotted regions indicate denticle bands, dotted lines the segmental boundaries. The regions missing in mutant larvae are indicated by the hatched bars. The transverse lines connect corresponding regions in mutant and normal larvae. Planes of polarity reversal in *runt* and *Krüppel* are indicated by the arrows. The two segment polarity loci *patch* and *gooseberry* are represented at the left. For indication of the polarity of the patterns, see Fig. 3. The patterns of *fused* and *ci*^D (not shown) look similar to the *gooseberry* pattern, whereas in *hedgehog* and *wingless* the deleted regions are somewhat larger, cutting into the denticle bands at either side. Four pair-rule mutants are shown in the centre. The interpretation of their phenotypes is based on the study of weak as well as strong alleles, combinations with *Ubx* (see text) and, in the case of *runt*, on gynandromorphs (unpublished). They probably represent the extreme mutant condition at the respective loci. The phenotypes of all known *barrel* and *engrailed* alleles (not shown) are somewhat variable and further studies are needed to deduce the typical phenotype. At the right, the two gap loci *Krüppel* and *knirps* are shown. Both patterns represent the amorphic phenotype as observed in embryos homozygous for deficiencies of the respective loci. The only known *hunchback* allele (not shown) deletes the meso- and metathorax.

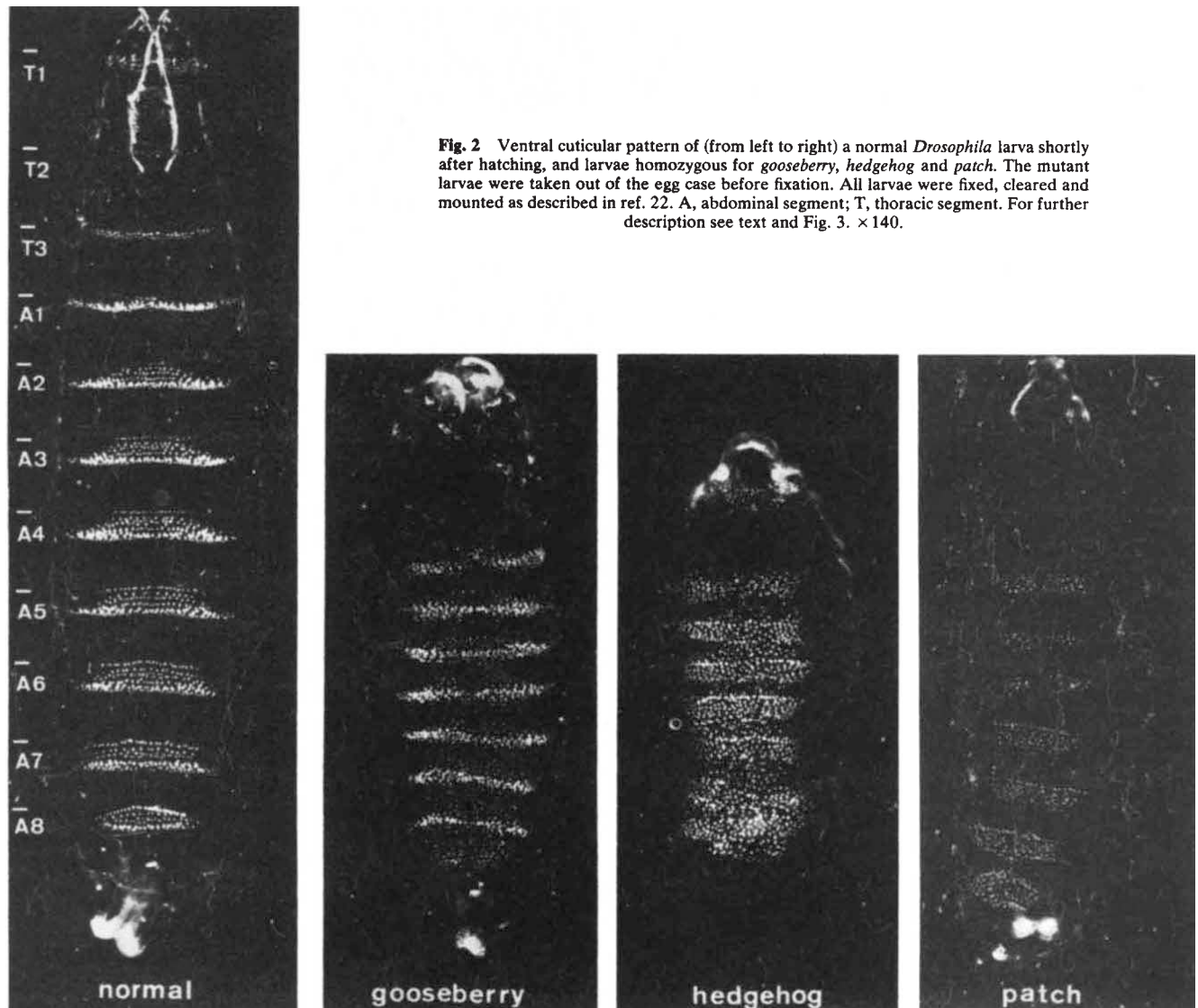


Fig. 2 Ventral cuticular pattern of (from left to right) a normal *Drosophila* larva shortly after hatching, and larvae homozygous for *gooseberry*, *hedgehog* and *patch*. The mutant larvae were taken out of the egg case before fixation. All larvae were fixed, cleared and mounted as described in ref. 22. A, abdominal segment; T, thoracic segment. For further description see text and Fig. 3. $\times 140$.

essentially from the deletion of the naked posterior part of the odd-numbered and the anterior denticle bands of the even-numbered segments (Figs 1, 4). The double segments thus formed are composites of anterior mesothorax and posterior metathorax, anterior first abdominal and posterior second abdominal segment, etc. The identification of the regions present or deleted in mutant larvae is based on the phenotypes produced by alleles with lower expressivity. In such embryos the deletions are in general smaller and variable in size. In a 'leaky' allele of *even-skipped*, the denticle bands of the even-numbered segments are frequently incomplete, whereas in *paired*² (*prd*²) the pattern deletions often involve only part of the naked cuticle of the odd-numbered segments, leading to a pairwise fusion of denticle bands (Fig. 4).

Further support for the composite nature of the segments in *prd* larvae was obtained in combinations with *Ultrabithorax* (*Ubx*), a homeotic mutation which when homozygous causes the transformation of the first abdominal segment into mesothorax⁵. In such *prd; Ubx* larvae, only the denticle band of the first double segment in the abdomen is transformed. The posterior margin of this band and the naked cuticle which follows remain abdominal in character and lack, for example, the typical mesothoracic sense organs. The composite nature of the segments in *paired* shows that the establishment of segmental identity does not require the establishment of individual segments as such.

The segmentation pattern in *prd; Ubx* larvae is typical of that of *paired* alone and is not affected by the *Ubx* homeotic trans-

formation. Similarly, in *prd; Polycomb* larvae the naked cuticle of alternating segments is deleted just as it is in *paired* alone, even though in *Polycomb* embryos all the thoracic and abdominal segments have an 8th abdominal character⁵. These combinations, and similar ones with *even-skipped*, indicate that the observed grouping of segments in pairs depends on the position of segments within the segmental array rather than the segmental identity. These combinations thus provide evidence that mutations such as *paired* and *even-skipped* affect different processes from those altered in homeotic mutants.

Other mutants in this class show different deletion patterns. The phenotype of *odd-skipped* is similar to that of *even-skipped*. However, in this case it is the odd-numbered denticle bands that are affected. In *odd-skipped*, the deleted region is smaller and is restricted to a more posterior part of the segment than in *even-skipped* (Figs 1, 4). *Barrel* has a phenotype similar to *paired* although the pattern is often less regular (Fig. 4). *Runt*, on the X chromosome, is the only pair-rule mutant showing mirror-image duplications. *Runt* embryos have half the normal number of denticle bands, each a mirror-image duplication of the anterior part of a normal band (similar to the duplications found in *patch*). The bands in *runt* embryos, as well as the region of naked cuticle separating them, are of unequal sizes (Figs 1, 4).

To the list of pair-rule loci we have added the *engrailed*¹⁰ locus. Lethal *engrailed* alleles¹¹ lead to a substantial deletion of the posterior region of even-numbered segments. In addition, the anterior margin and adjacent cuticle of each segment are

affected. Thus, the defect pattern in *engrailed* shows repeats which are spaced at both one- and two-segment intervals.

Each of the six different pair-rule loci affects a different region within a double segmental repeat. In no case does the margin of the deleted region coincide with a segment boundary. When the deleted region corresponds in size up to one entire segment (*paired*, *even-skipped*) it includes parts of two adjacent segments.

The phenotypes of the pair-rule mutants suggest that at some stage during normal development the embryo is organized in repeating units, the length of which corresponds to two segmental anlagen.

Gap mutants: one continuous stretch of segments deleted

One of the striking features of the mutations of the first two classes is that the alteration in the pattern is repeated at specific intervals along the antero-posterior axis of the embryo. No such repeated pattern is found in mutants of the third class and instead a single group of up to eight adjacent segments is deleted from the final pattern. Three loci have been identified which cause such gaps in the pattern (Table 1, Figs 1, 5). *Krüppel* (*Kr*) was originally described by Gloor¹². Embryos homozygous for *Kr* lack thorax and anterior abdomen. The posterior-terminal region with the abdominal segments 8, 7 and 6 is normal, although probably somewhat enlarged. Anterior to the 6th abdominal segment is a plane of mirror-image symmetry followed by one further segment band with the character of a 6th segment oriented in reversed polarity. The exact position of the plane of symmetry varies and does not usually coincide with a segmental boundary. A large part of the *Krüppel* pattern is reminiscent of the pattern observed in embryos produced by the maternal-effect mutant *bicaudal*, although no maternal

Table 1 Loci affecting segmentation in *Drosophila*

Class	Locus	Map position*	No. of alleles†	Ref.
Segment-polarity	<i>cubitus interruptus</i> ^D (<i>ci</i> ^D)	4-0	(2)	20
	<i>wingless</i> (<i>wg</i>)	2-30	6	9
	<i>gooseberry</i> (<i>gsb</i>)	2-104	1	This work
	<i>hedgehog</i> (<i>hh</i>)	3-90	2	This work
	<i>fused</i> (<i>fu</i>)‡	1-59.5	(9)	8, 20
	<i>patch</i> (<i>pat</i>)	2-55	8	This work
Pair-rule	<i>paired</i> (<i>prd</i>)	2-45	3	This work
	<i>even-skipped</i> (<i>eve</i>)	2-55	2	This work
	<i>odd-skipped</i> (<i>odd</i>)	2-8	2	This work
	<i>barrel</i> (<i>brr</i>)	3-27	2	This work
	<i>runt</i> (<i>run</i>)	1-65	1	This work
	<i>engrailed</i> (<i>en</i>)	2-62	6	11, 20
Gap	<i>Krüppel</i> (<i>Kr</i>)	2-107.6	6	12, 20
	<i>knirps</i> (<i>kni</i>)	3-47	5	This work
	<i>hunchback</i> (<i>hb</i>)	3-48	1	This work

* For the new loci (see last column) the map positions are based on recombination between the markers *S*, *Sp*, *Bl*, *cn*, *bw* for the second chromosome, and *ru*, *h*, *th*, *st*, *cu*, *sr*, *e*^S, *ca* for the third chromosome. For description of markers see ref. 20. The loci *runt*, *Krüppel* and *knirps* were further mapped using the breakpoints of deficiencies and duplications for the respective regions. All mutants were mapped by scoring the embryonic progeny of single recombinant males backcrossed to heterozygous females from the original mutant stocks.

† The numbers in parentheses refer to the alleles listed in Lindsley and Grell²⁰. All other alleles, except the *runt* allele, three *Kr* alleles and one *knirps* allele, were isolated in a screen for embryonic lethal mutants on the second chromosome. 5,800 balanced stocks were established from individual males heterozygous for an ethyl methane sulphonate-treated *cn bw sp* chromosome using the DTS-procedure suggested by Wright²¹. 4,500 of the stocks had one or more new lethal mutations. Unhatched embryos from 2,600 putative embryonic lethal stocks were inspected for cuticular abnormalities²². Third chromosomal mutants discovered in the second chromosomal balanced lines were recovered after selection through individual females by balancing individual third chromosomes over TM3. Complementation tests were carried out between mutants with similar phenotypes whereby the occurrence of mutant embryos among the progeny of the crosses served as the criterion for allelism. Three new *Kr* alleles were isolated in a screen for lethals over the original *Kr* of Gloor¹², and one *knirps* allele of presumably spontaneous origin was discovered on a TM1 chromosome. The *runt* allele was isolated in a screen for X-linked lethals.

‡ *fused* is a male-rescueable maternal-effect locus⁸. Thus, the segment polarity reversal is observed in *fu/fu* embryos from *fu/fu* mothers. The progeny of *fu/+* females show a normal embryonic pattern regardless of embryonic genotype.

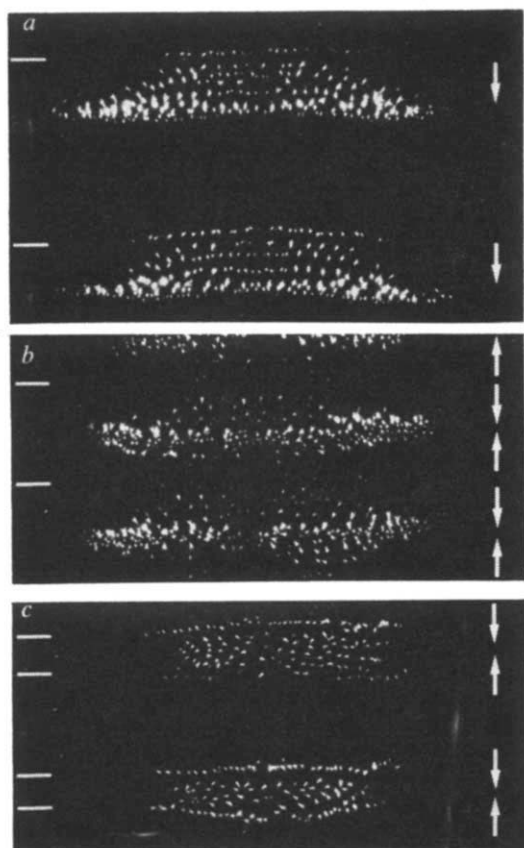


Fig. 3 Details from the ventral abdomen of a normal (a), a *gooseberry* (b), and a *patch* (c) larva. The positions of the segment boundaries are indicated at the left by the transverse lines. The arrows at the right indicate the polarity of the pattern as judged by the orientation of the denticles as well as the shape of the denticle bands.

component is involved in the production of the *Krüppel* phenotype (in preparation).

In the other two loci of this class, the gap in the pattern occurs in specific morphologically defined subregions of the larval pattern, in the thorax and in the abdomen, respectively. In *hunchback*, the meso- and metathoracic segments are deleted. In embryos homozygous for *knirps*, only two rather than eight denticle bands are formed in the abdomen. The posterior-terminal region including the 8th abdominal segment seems normal whereas the anterior abdominal denticle band is considerably enlarged. The anterior margin of the denticle band is morphologically similar to the first abdominal segment but combinations with *Ubx* show that the band is a composite with more than one segmental identity.

All three loci are required for a normal segmental subdivision of one continuous body region. The lack of a repeated pattern of defects suggests that the loci are involved in processes in which position along the antero-posterior axis of the embryo is defined by unique values.

When are genes affecting segment number active?

The phenotypes described above are observed only in homozygous embryos, indicating that the loci identified by these mutations are active after fertilization and are crucial for the normal segmental organisation of the embryo. We have described the mutations in terms of their effect on the differentiated pattern. However, in many instances their effect can be observed much earlier in development. In normal embryos segmentation is first visible 1 h after the onset of gastrulation as a repeated pattern of

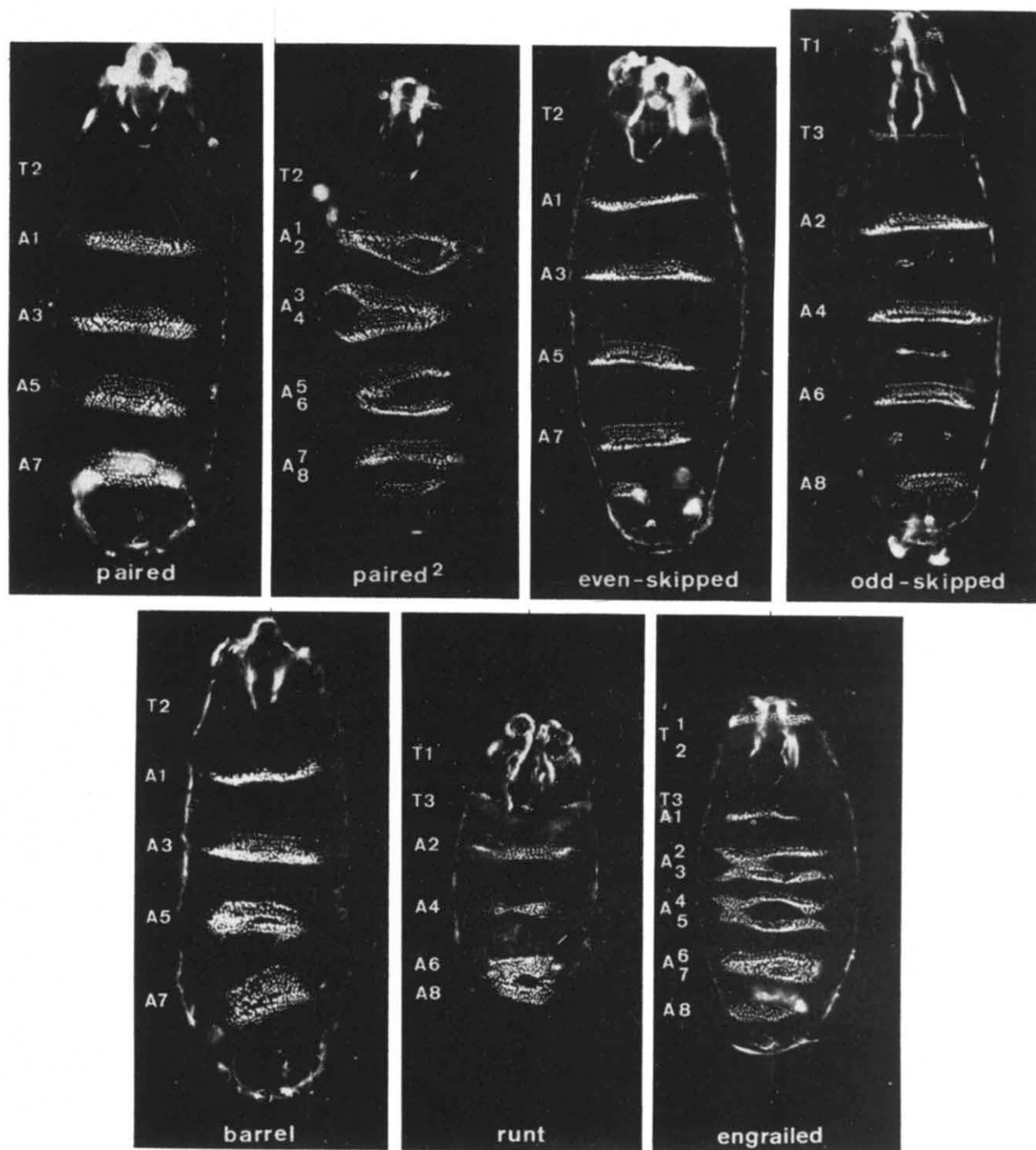


Fig. 4 Larvae homozygous for mutations at the six pair-rule loci. The segmental identity of the denticle bands is indicated at the left of each picture. A, abdominal band; T, thoracic band. For comparison with the normal pattern see Figs 1 and 2.

bulges in the ventral ectoderm (unpublished observations). In *paired* and *even-skipped* embryos the number of bulges is reduced and corresponds to the number of segments observed in the differentiated mutant embryos. *Krüppel*, *runt* and *knirps* embryos can be identified 15 min after the onset of gastrulation. All three mutations cause shorter germ bands, a phenomenon clearly related to the strong reduction in segment number observed in the differentiated larvae. Further evidence for an early activity at the *paired* locus was obtained using a temperature-sensitive allele. The extreme phenotype is only obtained when the embryo is kept at the restrictive temperature during the blastoderm stage. All these results indicate that the wild-

type genes defined by the mutations are active before the end of gastrulation during normal development.

Discussion

Segmentation in *Drosophila* proceeds by the transition from a single field into a repeated pattern of homologous smaller subfields. Mutant alleles at the 15 loci we have described interfere with this process at various points. Although each locus has its own distinct phenotype, we were able to distribute the mutations in three classes. In one class only a single large subregion of the embryo is affected, whereas in mutants of the other two classes a reiteration of defects is produced with a

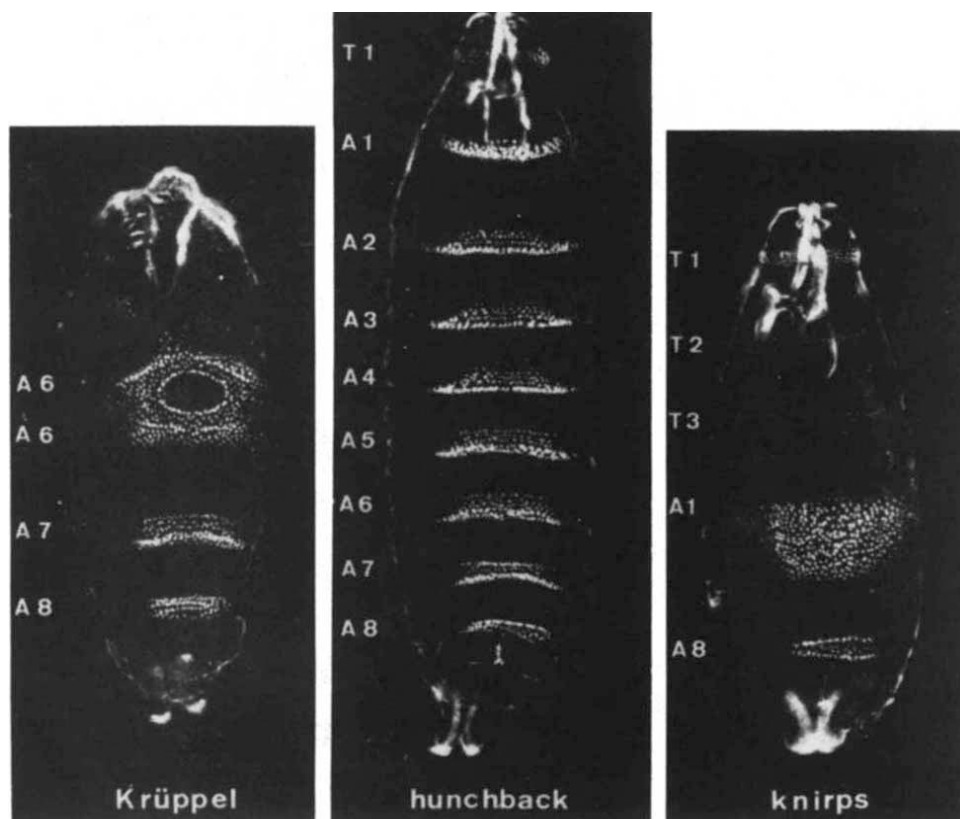


Fig. 5 Larvae homozygous for mutations at the three gap loci. A, abdominal segment; T, thoracic segment.

repeat length of one or two segments, respectively. This suggests that the process of segmentation involves three different units of spatial organization.

The organization of the egg is thought to be controlled by a monotonic gradient set up during oogenesis under the control of the maternal genome^{3,4}. All the mutants we have described depend on the embryonic rather than the maternal genome. None of them alters the overall polarity of the embryo, the head always being at the anterior and the telson at the posterior end of the egg. The most dramatic alterations of the pattern are produced in the gap mutants and involve one large subregion of the egg. *Hunchback* and *knirps* affect the development of thorax and abdomen, respectively, and might be involved in the establishment of these large morphologically unique subregions of the embryo. The large mirror-image duplications found in the posterior pattern of *Krüppel* embryos are similar to those of *bicaudal*. The *bicaudal* phenotype has been interpreted as resulting from an instability in the maternal gradient^{3,13}, and thus *Krüppel* might be involved in the maintenance or elaboration of this gradient in the posterior egg region after fertilization.

The smallest repeat unit, the individual segment, is affected in mutants at the six segment-polarity loci. The pattern alteration consists of a mirror-image duplication of part of the normal pattern with the remainder deleted. In these mutants the deleted region corresponds in size to more than half a segment. Mutations causing smaller deletions in the segmental pattern do not lead to polarity reversals (unpublished observations). The mirror-image duplications produced by the pair-rule mutation *runt* are also associated with a deletion of more than half a repeat unit, that is, more than one segment. The tendency of partial fields containing less than half the positional values to duplicate is well described for imaginal disks in *Drosophila*¹⁴, as well as for the larval epidermis in other insects^{15,16}. On the other hand, the same types of pattern duplication are also produced in conditions which do not involve cell death and regeneration, but rather a reorganization of the positional information in the entire field^{3,17}. More detailed studies of early development in mutant embryos may reveal which mechanism is responsible for the different mutant phenotypes.

Given the evidence for a homology between segments, the existence of a class of mutants affecting corresponding regions in each individual segment is perhaps not surprising. The discovery of a mutant class affecting corresponding regions in every other segment was not expected and suggests the existence at some time during development of homologous units with the size of two segmental anlagen. It is possible that the double segmental unit corresponds to transitory double segmental fields which are established early during embryogenesis and are later subdivided into individual segments. At the blastoderm stage the epidermal primordium giving rise to thorax and abdomen is only about 40 cells long¹. An initial subdivision into double segments would avoid problems of accuracy encountered in a simultaneous establishment of segment boundaries every three to four cells. A stepwise establishment of segments implies that the borders defining double segments be made before the intervening ones. The mutant phenotypes do not definitely show which, if any, of the segment borders define a primary double segmental division in normal development. The mutant phenotypes which come closest to a pattern one would expect if the transition from the double segment to the individual segment stage were blocked are the patterns of *paired* and *even-skipped*. Both suggest the frame meso- and methathorax, 1st and 2nd, 3rd and 4th abdominal segment, etc.

It is also possible that the double segmental units are never defined by distinct borders in normal development. The existence of a double segmental homology unit may merely reflect a continuous property such as a wave with a double segmental period responsible for correct spacing of segmental boundaries (see, for example, refs 18, 19). We have not found any mutations showing a repeat unit larger than two segments. This may indicate that the subdivision of the blastoderm proceeds directly by the double segmental repeat with no larger intervening homology units. However, the failure to identify such larger units may reflect the incompleteness of our data.

Drosophila has been estimated to have about 5,000 genes and only a very small fraction of these when mutated result in a change of the segmental pattern of the larva. Some of the loci

described here were known previously but only in the case of *Krüppel* has the embryonic lethal phenotype been recognized as affecting segmentation.¹² The majority of the mutants described here have been isolated in systematic searches for mutations affecting the segmentation pattern of the *Drosophila* larva. These experiments are still incomplete. Most of the alleles on the second chromosome were isolated in one experiment which yielded an average allele frequency of four or five alleles per locus (based on 42 embryonic lethal loci). From this yield and similar calculations for the third and first chromosomes, we estimate that we have identified almost all segmentation loci on the second chromosome and about 50% each of those on the third and first chromosome. Our sample of 15 loci should therefore represent the majority of the loci affecting segmentation in the *Drosophila* genome. Thus, in *Drosophila* it

would seem feasible to identify all genetic components involved in the complex process of embryonic pattern formation.

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Note added in proof: All known *barrel* alleles fail to complement *hairy*²⁰, suggesting that the *barrel* mutations are alleles at the *hairy* locus.

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Chemical synthesis of a polypeptide predicted from nucleotide sequence allows detection of a new retroviral gene product

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We previously determined the nucleotide sequence of the 3' end of Moloney leukaemia virus and discovered the potential coding region for an unknown protein, R. We now show that this region does encode a protein. A pentadecapeptide of R was chemically synthesized and antibodies raised against it. Antisera to the synthetic peptide recognize the R protein and the env precursor polyprotein in infected cells. The strategy presented here should provide a general method for accessing proteins predicted by nucleotide sequences.

THE accepted genetic structure of replication-competent murine leukaemia viruses, such as Moloney, Rauscher, Friend and AKV, includes three genes. These genes, *gag*, *pol* and *env*, are arranged, in respective order, along the single-stranded RNA genome. They are transcribed from the integrated double-stranded DNA provirus¹. The protein products of these three genes are understood in considerable detail. The *gag* gene encodes a polypeptide of about 65 kilodaltons which is proteolytically processed to proteins, amino to carboxy terminal, of 15, 12, 30 and 10 kilodaltons, respectively². These proteins are found in the core of the virion, and one, p30, has been associated with virus tropism³. The second gene, *pol*, is expressed as an apparent extension of the *gag* gene such that an approximately 180-kilodalton polyprotein containing both *gag* and *pol* components is observed. The polyprotein is processed to a 70-kilodalton protein which is the reverse transcriptase⁴. This enzyme is responsible for copying the single-stranded RNA genome of an infecting virus into the double-stranded DNA structure which can integrate into host DNA. The third gene, *env*, encodes a polyprotein which is glycosylated and processed into components gp70 and p15E (ref. 5). gp70 is the major envelope component and determines viral host range. It is sometimes found disulphide linked to p15E, a hydrophobic

protein which may anchor gp70 to viral and cellular membranes⁶. Messenger RNA molecules have been described which can account for the expression of these three genes⁷.

To understand fully the genetic structure of this class of viruses, we have been analysing their nucleotide sequences. We began our study at the 3' end of Moloney murine leukaemia virus (Mo-MuLV) because of our particular interest in the *env* gene which was thought to be the most 3'-proximal gene. Surprisingly, we found an open reading frame which extended beyond the coding region for known viral products⁸. Even though we then had no evidence for a gene product, we provisionally named this the R-region because it is the coding region that is the furthest to the right in the genome. Here we report the detection of the R product. We chemically synthesized part of the R protein, raised antibodies to the synthetic peptide and detected immunologically cross-reactive material in infected cells.

The DNA sequence

For orientation purposes, several features of the previously reported⁸ DNA sequence are presented here (Fig. 1). The DNA sequence was initially generated from an 1,108-base pair cDNA clone obtained by cloning the product of reverse transcription of

purified detergent-disrupted Mo-MuLV. We have also sequenced most of this region of a full-length virus clone that can be demonstrated by transfection studies to carry all biologically active Mo-MuLV coding sequences. This new DNA sequence agrees with the original except for the differences described in Fig. 1 legend. We consider these differences to represent either variability in the population of biologically active genomes or artefacts due to the slight 'unfaithfulness' of the reverse transcriptase reaction (unpublished observations) which generated the clone we initially examined.

Proceeding from 5' to 3' along the DNA strand corresponding to genomic RNA, we see: the coding region for most of p15E (the carboxy-terminal *env* coding region); the origin of second-(positive) strand DNA replication; the inverted repeats, IR_L and IR_R, which flank the portion of viral sequences that are duplicated at the 5' and 3' ends of the integrated provirus (the so-called long terminal repeats, LTRs) and are responsible for the structure of the DNA of the virus resembling bacterial transposons^{8,9}; and the poly(A) addition signal and presumptive 3' ultimate nucleotides preceding the poly(A) tail⁸. Also indicated are features which are active at the 5' copy of the LTR—the putative promoter for genomic expression and the first transcribed base⁸.

A new protein predicted by DNA sequence

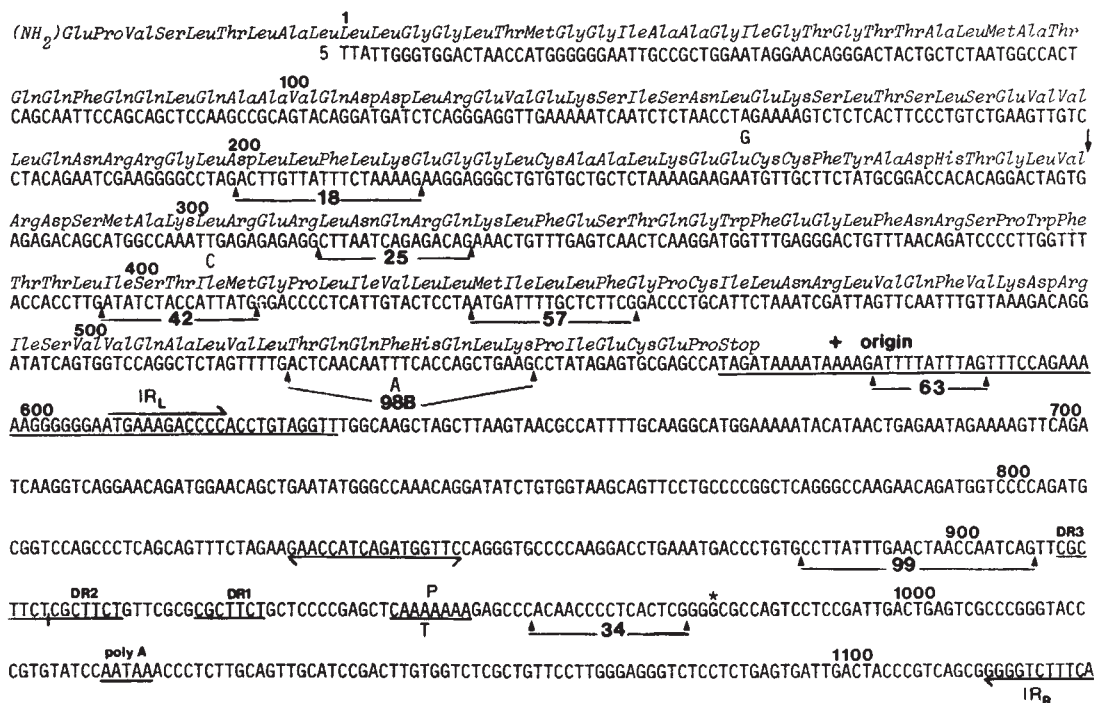
The new genetic region that we found can best be understood by considering what was previously thought to be the right-most coding region of the provirus—that for the viral protein p15E. The amino terminus of p15E can be positioned precisely by overlap between the protein sequence predicted by our DNA sequence and the protein sequence obtained by Copeland and Oroszlan (personal communication) (Fig. 1). The carboxyl terminus of p15E is not yet precisely defined. The apparent molecular size of p15E as determined on SDS gels is about 15 kilodaltons, a size estimate compatible with the mobility of a hydrophobic protein of about 100 residues. Surprisingly, we do not find a translational termination codon in this region. Instead, the open translational reading frame extends for a total of 196 triplets before encountering a stop. Thus we suspected that the

primary *env* gene protein product in fact contained three peptides—gp70, p15E and the newly identified protein, R (positions ~100–196 in Fig. 1). Therefore, we looked for the R protein.

Chemical synthesis and detection of the R protein in infected cells

To detect and determine the cellular location of the R product, several peptides of the predicted Mo-MuLV R protein were synthesized by solid-state methods¹⁰ and antibodies raised to these synthetic peptides. The predicted C-terminal peptides were synthesized because the N-terminus is not precisely known. Specifically, the C-terminal 15 residues (Leu-Thr-Gln-Gln-Phe-His-Gln-Leu-Lys-Pro-Ile-Glu-Cys-Glu-Pro) were attached to the carrier molecule keyhole limpet haemocyanin (KLH) and injected into six rabbits and four mice. We assayed the sera for the ability to precipitate immunologically a 36-residue synthetic substrate containing the 35 C-terminal residues of the R protein preceded by ¹²⁵I-tyrosine (¹²⁵I-Tyr-Ile-Leu-Asn-Arg-Leu-Val-Gln-Phe-Val-Lys-Asp-Arg-Ile-Ser-Val-Val-Gln-Ala-Leu-Val-Leu-Thr-Gln-Gln-Phe-His-Gln-Leu-Lys-Pro-Ile-Glu-Cys-Glu-Pro). Sera from all the immunized animals showed a positive response of 10–70-fold over normal serum (Table 1). We then looked for the R protein in lysates of MuLV-producing SCRF 60A cells that had been labelled by a 2-h ³⁵S-methionine pulse (the R protein contains no tyrosine and so was not expected to label with iodine). As can be seen in Fig. 2, an anti-R serum detects a protein with an apparent molecular size of approximately 80 kilodaltons in infected cells. All the individual immune rabbit and mouse sera detected proteins with the same molecular size. Additionally, no target molecules were observed in uninfected mouse lymphocytes, but the same band was seen after infection with Mo-MuLV (not shown). Because the nucleotide sequences showed that p15E and R were in the same reading frame, we expected that this molecule was, in fact, the *env* precursor. Antibodies to gp70, including an anti-gp70 hybridoma, detect a molecule with the same apparent molecular size as that precipitated by anti-R,

Fig. 1 Nucleotide sequence of the 3' end of the Mo-MuLV provirus. The sequence of the 1,123-nucleotide plus-strand from the 3' LTR and carboxy-terminal virus coding region was determined from an 1,108-base pair long cDNA clone⁸ and extended slightly by sequence from an infectious clone. The four differences in the infectious clone are indicated below the running sequence. The protein sequence translated from the DNA appears above the nucleotide sequence. The first 34 amino-terminal residues were determined by Copeland and Oroszlan and positions 10–34 match our sequence. The arrow at the end of the third line indicates the region of the carboxy terminus of p15E. The rest of the amino acid sequence is that of the R protein. The numbered oligonucleotides (25, 42, 57 and 98B) represent those corresponding to AKV virus and are shown in detail in Fig. 6. The underlined region downstream from the R coding region is the origin of plus-strand DNA synthesis. The regions marked IR_L and IR_R are the inverted terminal repeats which flank the LTRs. Within the LTR we recognize a 17-base long palindrome (underlined) and three direct repeats (DR 1, 2 and 3) which lie just upstream from the promoter Hogness box (p) for the 5' end of the viral transcript (*). Further down the molecule is the poly(A) addition signal for the 3' end of the transcript.



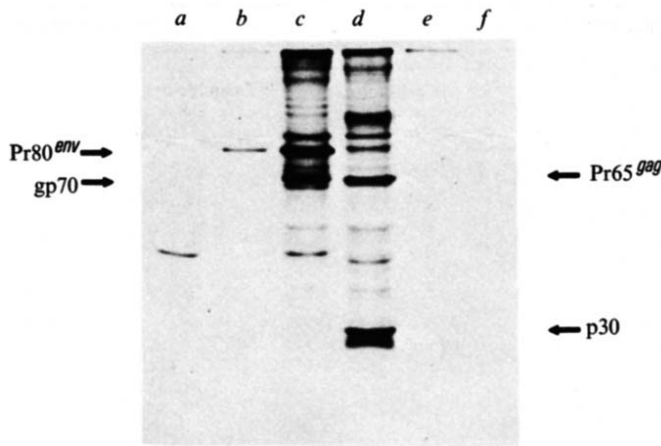


Fig. 2 Antibody to synthetic R peptide precipitates Pr80^{env} . Log-phase SCRF 60A cells, which produce a virus indistinguishable from Mo-MuLV¹⁸, were grown in Eagle's minimal essential medium (MEM) with 10% fetal calf serum. The cells were labelled at 37 °C at 2×10^6 cells ml^{-1} for 2 h with ^{35}S -methionine (940 Ci mmol^{-1} , 100 $\mu\text{Ci ml}^{-1}$) in Hank's balanced salts with 10% Eagle's MEM. Cells were chilled, washed with 0.15 M NaCl, 10 mM sodium phosphate pH 7.5, extracted with 0.15 M NaCl, 10 mM sodium phosphate pH 7.5, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 2% Trasylol for 20 min at 0 °C, then sonicated and spun at 12,000g for 5 min. The lysate was pre-cleared by reacting with 20 μl of normal rabbit serum and 20 μl of normal goat serum for 30 min at 0 °C, followed by the addition of formalin-fixed *Staphylococcus aureus* for 30 min and centrifugation for 15 min at 12,000g. Portions of the lysate were reacted with 5 μl of normal rabbit (a), rabbit anti-synthetic R pentadecapeptide (b), goat anti-gp70 (c), goat anti-p30 (d), normal goat sera (e), or anti-gp70 hybridoma (no. R1-16G07, ref. 19) (f) culture media for 1 h at 0 °C. Complexes were collected with *S. aureus* and after two washes with 500 mM LiCl, 100 mM Tris, pH 8.5, dissolved in loading buffer cleared of *S. aureus*, and loaded on an 11% SDS-polyacrylamide gel. The gel was soaked in Enhance (NEN) for 90 min, H_2O for 60 min, dried and exposed to film. The bands indicated have been identified previously in this laboratory and by others²⁰.

confirming that we are observing the *env* precursor. To rule out the possibility that these were two distinct molecules with a fortuitous correspondence in molecular size, we did two experiments. First, when the lysates are pre-cleared with anti-R sera, the 80-kilodalton radioactive target for anti-gp70 sera is removed. Conversely, clearing the lysate with anti-gp70 sera removes the R determinants. Second, proteolytic peptide fingerprinting of the two 80-kilodalton molecules precipitated by anti-R and anti-gp70 shows them to be identical (Fig. 3). Thus, we have identified the 80-kilodalton band as the complete *env* gene polyprotein product containing gp70, p15E (as shown by others) and R determinants.

Using different gel electrophoresis conditions, we were able to detect a protein of estimated molecular size 12 kilodaltons specifically precipitated by anti-R serum (Fig. 4). We tentatively identify this small protein as R although positive identification awaits N-terminal amino acid analysis. Also, we have observed, by indirect immunofluorescence, R protein determinants on the plasma membrane of SCRF 60A cells (to be published elsewhere), a result made more significant by the fact that the anti-R serum does not precipitate gp70 or p15E. Our experiments do not indicate at what time the p15E-R cleavage occurs and it is possible that the true gp70 membrane anchor is the 22-kilodalton protein containing p15E and R peptides. In that case, the proteolytic cleavage which generates p15E and R occurs during their isolation. We have not observed a 22-kilodalton protein with R determinants.

The R gene is present in another virus

Pederson and Haseltine¹¹ determined the nucleotide sequences of the large ribonuclease T₁ fragments of AKV virus, an endogenous leukaemia virus of AKR mice. By computer search we identified several of these fragments as having partial homologies to skeins of our Mo-MuLV sequence. Our defined order

makes a reasonable match with the order obtained experimentally by Pederson, Crowther and Haseltine (personal communication). The matches within the R-region are indicated in Fig. 1 and detailed in Fig. 5. From these alignments we note first the matched fragments are not wholly conserved, which perhaps warns us as to what to expect from future comparisons of sequences of related viruses, and second, that matches within the R-region indicate that AKV probably encodes an R protein much like that of Mo-MuLV. Specifically, (1) none of the AKV differences introduces nucleotides which indicate a premature translational stop, (2) several (9) AKV/Mo-MuLV nucleotide differences are in third positions in triplets and do not alter the coded protein sequence, (3) some nucleotide differences do change R-protein amino acids, but the replacements tend to be conservative or synonymous (as indicated in Fig. 5), such as phenylalanine to tyrosine in spot 98B, Asn-Gln-Arg to Lys-Gln-Gln in spot 25, or Met to Leu in spot 57. All these substitutions are rated as positive correlations by the Dayhoff mutation matrix¹³. Additionally, a preliminary sequence obtained by Herr (personal communication) through 211 nucleotides of the R-region of AKV shows an open reading frame containing 51 nucleotide differences with our Mo-MuLV sequence. These 51 differences make only six amino acid alterations, all of which are synonymous. These observations indicate that the R-protein sequence is highly conserved in the two viruses.

Comments about the R protein

The entire amino acid sequence of the R protein does not correspond closely to any protein sequence in ref. 12. Some subregions of R, however, are reminiscent of subregions of known proteins such as dehydrogenases and proteases, perhaps

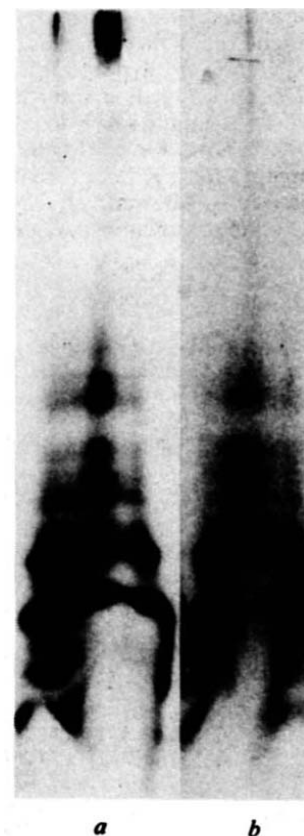


Fig. 3 Pr80^{env} precipitated by anti-R and anti-gp70 have the same one-dimensional peptide maps. ^{35}S -methionine-labelled extracts of SCRF 60A cells were prepared, reacted with anti-R (a) or anti-gp70 (b) sera, and analysed as described in Fig. 2 legend. The 80-kilodalton band precipitated by each serum was cut from the dried gel, and mapped by limited proteolysis with *S. aureus* V8 protease using the techniques of Cleveland *et al.*²¹. The reaction products were electrophoresed on a 5–17% polyacrylamide gel.

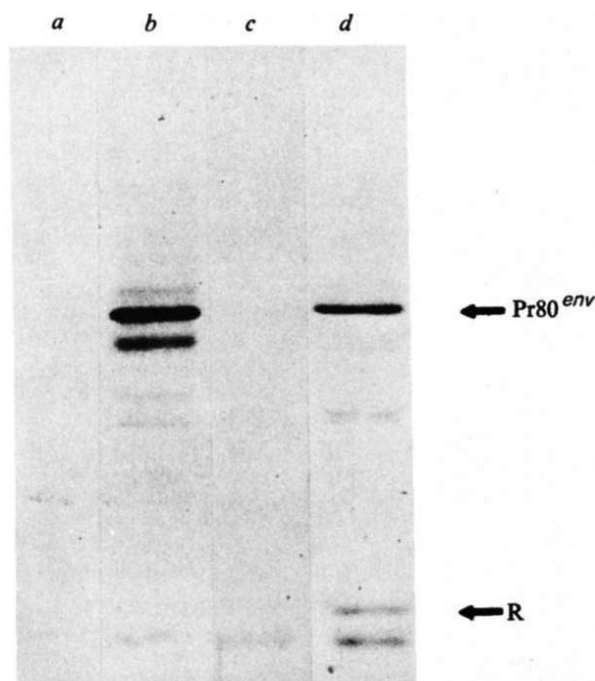


Fig. 4 Detection of the R protein. A ^{35}S -methionine-labelled lysate of SCRF 60A cells (as described in Fig. 2 legend) was divided into four equal portions and immunologically precipitated with normal goat (a), goat anti-gp70 (b), normal rabbit (c) or rabbit anti-R (d) serum, collected with *S. aureus* and electrophoresed on a 5–17.5% SDS-polyacrylamide gradient gel. The lower band in lanes c and d is usually seen with any normal rabbit serum and is thus not considered specific.

implicating domains of the R protein in nucleotide binding or protein–protein interactions.

The R protein might function simply as a structural viral protein. In this regard, the very hydrophobic domain between positions 32 and 61 suggest that R is inserted into cellular or viral membranes where it might serve to anchor other viral proteins. As mentioned above, we already have evidence from fluorescence microscopy that R is present in the plasma membranes of infected cells. Alternatively, the R protein might be involved in other sorts of functions. As originally noted by

Fragment	Mo-MuLV AKV
25	ArgLeuAsnGlnArgGln GCTTAATCAGACAGAC GCTAAACAACAACAG LeuLysGlnGlnGln
42	LeuIleSerThrIleMet GATATCTACCATTATG GATATCCACCATCATG IleSerThrIleMet
57	LeuMetIleLeuLeuPhe AATGATTTTGCTCTTCG GTTAATTTTACTCTTTG LeuIleLeuLeuPhe
98B	LeuThrGlnGlnPheHisGlnLeuLys GACTCAACAATTCACAGCTGAAG GACTCAACAATATCATCACTTAXG ThrGlnGlnTyrHisGlnLeu

Fig. 5 Comparison of the R gene in two viruses. The oligonucleotide sequences determined by Pederson and Haseltine¹¹ for AKV were matched by computer to our Mo-MuLV sequence. Those matches within the R-region are shown, Mo-MuLV above AKV, with the corresponding translated amino acids. The numbers in the margin are those used by Pederson and Haseltine. Differences between the nucleotides are underlined. In fragment 98B, the phenylalanine codon in our cDNA sequence was a tyrosine codon in the infectious clone and is a tyrosine in AKV. The X in fragment 98B is an unsolved position for AKV¹¹.

Table 1 Precipitation of ^{125}I -synthetic peptide by sera from R-KLH-immunized rabbits and mice

	Immunization schedule	^{125}I -peptide precipitated (c.p.m.)
Rabbit		
1	None	432
2	a	4,867
3	a	15,359
4	b	21,200
5	b	18,436
6	c	5,548
7	c	19,799
Mouse		
1	None	553
2	None	521
3	d	18,522
4	d	20,282
5	d	28,890
6	d	38,497

The carboxy-terminal R pentadecapeptide (LTQOFHQLKPIECEP) was prepared and its acid hydrolysate was shown to be of correct composition. It was shown to be pure by TLC on both silica and cellulose in $n\text{BuOH/EtOAc/HOAc/H}_2\text{O}$ (1:1:1:1) and electrophoresis in pyridine acetate, pH 5.6, on Whatman 3MM. The sample was visualized by reaction with ninhydrin reagent. It was conjugated to KLH through the cysteine sulphhydryl. 63 μl of 15 mg ml^{-1} *m*-malimidobenzoyl-*N*-hydroxysuccinimide ester^{16,17} in *N,N'*-dimethylformamide was added dropwise with stirring to KLH (10 mg ml^{-1}) in 10 mM potassium phosphate, pH 7.0. After stirring for 30 min, the mixture was filtered and applied to a Sephadex G-25 column (0.1 M phosphate, pH 6.0). The activated protein (2.3 ml) was mixed with 0.1 ml of the R pentadecapeptide (10 mg ml^{-1} in 0.1 M potassium phosphate, pH 7.3, 5 mM EDTA) and the solution adjusted to pH 6.5, stirred for 4 h at room temperature and chromatographed on Sephadex G-100 (0.1 M ammonium bicarbonate, pH 9.5). The conjugated protein was collected and used directly for immunization of female New Zealand White rabbits (1.5–2.5 kg) or adult female G_{128} mice (except mouse 1, which was BALB/c) as follows: Rabbits: 1 ml to hind footpads and subcutaneously at multiple sites along the back. Schedule a: 1 mg per kg in complete Freund's adjuvant (CFA, 1:1), days 0, 7 and 14; bleed days 21 and 28. Schedule b: 200 μg per rabbit in CFA on day 0; 50 μg per rabbit in incomplete Freund's adjuvant (ICF, 1:1) on day 7; 50 μg per rabbit in alum (4 mg per rabbit) intraperitoneally on day 14; bleed days 21 and 28. Schedule c: as schedule b except initial injection was 50 μg per rabbit. Mice were immunized intraperitoneally (schedule d) with 35 μg in CFA (1:1) on day 0; 35 μg with 500 μg alum intraperitoneally, day 14; bleed day 21. ^{125}I -36-mer (5×10^6 c.p.m.) in immune precipitation buffer (0.15 M NaCl, 10 mM sodium phosphate pH 7.5, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 2% Trasyol) was incubated with 5 μl serum for 1 h at 0 °C, with formalin-fixed *S. aureus* for 30 min, then centrifuged for 15 min at 12,000g. Pellets were washed once in buffer, twice in 500 mM LiCl/100 mM Tris pH 8.5, then counted. *S. aureus* in the absence of serum bound, 528 c.p.m.

Fisher¹⁴, genes and their regions of action seem to cluster. The R-region lies between the p15E coding region and the origin of positive-strand replication and LTR. It is, therefore, possible that R functions in replication or transposition (integration).

On the other hand, the R protein could have a role in viral transformation. A problem of the leukaemia viruses has been that, although they transform lymphoid tissues, they carry no apparent transforming genes. As a transforming protein, R could act directly or could interact with another protein. In either case, two important points must be explained. First, although they replicate in virtually all cell types of the mouse, these viruses only transform specific cells. Second, changes in the gp70 protein, which is encoded upstream from the R protein, are highly correlated with leukaemogenicity of various viruses, notably those called MCF (ref. 15). Therefore, we imagine either that gp70 and R interact directly (perhaps through disulphide linkage) and have, in the Moloney case, a T-cell-specific action or that gp70 triggers some cell-type-specific event which renders the T cell sensitive to the leukaemogenic effect of the R protein. What would cause the virus to maintain this gene is unknown.

The finding that Moloney and AKV, two viruses which cause T-cell lymphomas, have closely related R proteins, does not clarify the problem of specificity. We are now studying the R-region of other viruses such as Friend erythroleukaemia virus, which causes a disease of a different cell type, and a non-pathogenic xenotropic virus to determine whether the R protein is related to the pathogenicity and/or target tissues of the viruses.

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LETTERS

An extraordinary new celestial X-ray source

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The discovery of an extraordinary new X-ray source detected during a guest observer programme carried out with the Einstein X-ray Observatory is reported here. The object, designated G109.1–1.0, was found using the imaging proportional counter detector (IPC) on 17 December 1979. The IPC is sensitive to X-ray photons with energies between 0.1 and 4.5 keV. A longer exposure (3.6×10^3 s) IPC picture of a 1° field of view centred on the SNR was obtained on 8 July 1980 and is shown in Fig. 1. The picture shows a clear semicircular arc with an angular diameter of 36 arc min which appears to be the outer shell of the SNR. The resolution of the picture is 1 arc min. At the exact centre of the curvature of the shell is a strong compact source which we designate GF2259+586. The X-ray coordinates of GF2259+586 are RA = 22 h 59 min 02.5 s and dec = $58^\circ 36' 38''$. The 1 arc min accuracy of the coordinates is limited by systematic calibration errors in the IPC detector. Emerging from the east side of the central source is a jet-like structure which curves northwards in another continuous arc smoothly joining the outer shell. On closer inspection, the jet appears to bifurcate with a second component continuing radially outwards from the central compact source. This very unusual structure has parallels with the W50 SNR with its central X-ray source SS433 and jet (F. D. Seward, personal communication).

The western half of the circular shell appears to be completely absent with the exception of a weak X-ray source located at 19 arc min from the central source and coincident in position with the bright Sharpless H II region¹ S152, which has an angular diameter of 2 arc min. The S152 X-ray source does not show up in Fig. 1 because of shadowing by a detector support rib, but does appear in the earlier exposure of 12 December. We note that S152 and GF2259+586 are accurately aligned along the axis of the X-ray jet. The Palomar red and blue prints fail to show any nebulosity associated with the X-ray shell. There are, however, two very faint filaments each ~ 2 arc min long located well inside the southern part of the shell at a distance of 12–13 arc min from the central source. Within the 1 arc min (radius) X-ray error circle of GF2259+586 there are about 50 possible

optical counterparts to the limit of the Palomar red print, the brightest of which has a V magnitude of ~ 15 . Also on the western side, adjacent to S152, is a second Sharpless H II region S153, which has an angular diameter of 5 arc min and is 17 arc min from the central X-ray source.

Radio emission was first detected² from this region (CTB 109)² in 1960 at 960 MHz. Their measured flux density of 75 Jy combined with a later integrated flux density measurement of 40 Jy at 3,200 MHz by RaghavaRao *et al.*³ indicate a nonthermal spectrum. The value of the spectral index $\alpha = -0.52$ (S proportional to ν^α) is typical of a SNR. One of us (P.C.G.) has obtained a differential radio map of this region at 6 cm with the NRAO 91-m telescope as part of a survey for variable sources in the galactic plane. The 6-cm map corresponds fairly closely with the X-ray emission including the jet-like feature but unfortunately there is a gap in the observations at the location of the central X-ray source. A scan through this location at 2.8 cm in June 1980, with the Algonquin Radio Observatory 46-m telescope, failed to detect a discrete radio source to a limit of 100 mJy. The 6-cm radio data indicate that the jet-like feature continues for a short distance west of the central source. There also seems to be emission extending to the north-west and there is strong radio emission from S152 and weaker emission from S153. On the western side there is no clear radio evidence for the continuation of the circular shell. The source has also been mapped at Westerbork at 50 cm. (V. A. Hughes, personal communication).

Although the true nature of the source is not entirely clear, certain aspects of the morphology suggest we may be dealing with a SNR. If so, a distance may be obtained by using the relationship of Clark and Caswell⁴ between radio surface brightness and distance. The derived 408-MHz surface brightness (Σ) of $1.4 \times 10^{-20} \text{ W m}^{-2} \text{ Hz}^{-1} \text{ s}^{-1}$ using an angular diameter of 36 arc min falls close to a change in the slope of the Σ - D relationship. From their equations we arrive at a distance of 3.6 ± 0.4 kpc. Note that Crampton *et al.*⁵ obtained a distance of 3.6 kpc for S152 and a distance of 4 kpc for S153. On the Palomar blue print S152 exhibits a curved tail which extends back towards GF2259+586. This and the alignment mentioned above suggest a possible relationship between the two objects. Hence the distance estimate is not dependent entirely on the SNR assumption.

At a distance of 3.6 kpc, the linear diameter of the shell is 38 pc, comparable to the Cygnus Loop and the Vela SNR⁶.

A low energy cutoff is observed in the X-ray spectrum obtained from the IPC pulse height analyser (PHA) as expected for a ~ 3.6 -kpc distance. However, actual spectral fits must await further IPC calibration analysis. One estimate of the X-ray absorption can be obtained from the reddening of the central stars of S152 and S153 which have $E(B-V) = 1.25$ and 0.75

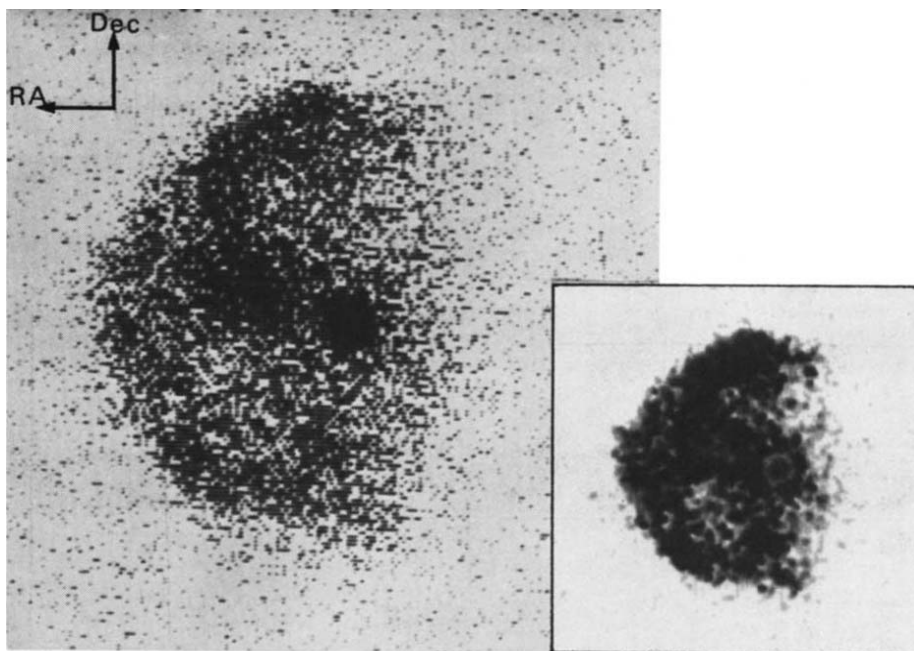


Fig. 1 IPC picture of a 1° field of view centred on G109.1-1.0. The inset shows the central core of the same object with better definition.

respectively⁵. These values can be converted to column densities of 0.85 and $0.51 \times 10^{22} \text{ cm}^{-2}$, respectively, using the relationship of Ryter *et al.*⁷. We regard these as upper limits because of the possibility of dust in the H II regions themselves. Adopting a value of $N_{\text{H}} = 0.3 \times 10^{22} \text{ cm}^{-2}$, we estimate that the luminosity of the SNR, excluding the central source, is $2.7 \times 10^{35} \text{ erg s}^{-1}$. If N_{H} is as high as $0.5 \times 10^{22} \text{ cm}^{-2}$ the corresponding luminosity is $4.9 \times 10^{35} \text{ erg s}^{-1}$. Applying the theory of adiabatic blast waves as described in ref. 6, we find that the age of SNR is $\sim 12,000$ yr. The ambient interstellar density is $\sim 0.1 \text{ cm}^{-3}$ and the energy released in the supernova explosion is $3.8 \times 10^{50} \text{ erg}$. The current shockwave velocity is predicted to be 600 km s^{-1} . The derived parameters are very similar to the Cygnus Loop and Vela SNR. By analogy with the Cygnus Loop we expect that the optical filaments noted earlier will have a much lower velocity than the predicted shock velocity.

If indeed this is a SNR, it is unusual because of its strong compact central source and jet-like feature which emerges on the eastern side of the central source. The PHA spectrum of the central source appears harder than the spectrum of the shell. Assuming thermal emission with $kT = 4 \text{ keV}$, the corresponding luminosity of the central source is $3 \times 10^{34} \text{ erg s}^{-1}$. The luminosity is uncertain by a factor of two but is of the same order as the X-ray emission from SS433. The most striking characteristics of this source are: (1) the semicircular shell which suggests a SNR; (2) a compact central X-ray source; and (3) a large-scale jet. These are also conspicuous features of the unusual object SS433 and its associated SNR, W50. Consequently, these two objects may be members of a new class of SNR. On the other hand, there are obvious differences in the morphology of the two objects. The spiral-like structure of the conspicuous X-ray jet and the hint of a similar feature to the south suggest that we may not be observing a SNR shell at all. The appearance may be the result of two jets which are being ejected from the central source and wound by rotation to give a shell-like structure. Alternatively, large-scale magnetic fields may be responsible for the observed geometry.

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Note added in Proof: One of us (P.C.G.) recently obtained VLA observations of the source at 20 cm and 6 cm. A weak compact radio source was found 2 arc min from GF2259+586 but there is no optical counterpart to the limit of the Palomar Sky Survey.

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Diffuse X-ray emission from the jets of SS433

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The discovery of strong, variable emission lines¹, which are Doppler-shifted² from SS433, led to the development of a kinematic beam model³⁻⁵ which explains the principal features of the time varying optical spectrum. In this model two opposed jets of material moving outwards from the central star at $0.258c$ precess with a period of 164 days, about an axis inclined 80° (or 20°) to the line of sight of the observer. The locus of the two beams forms a cone with an opening half angle of 20° (or 80°) (in the model an ambiguity exists between the inclination and cone angles, as the optical data alone cannot distinguish between the two possibilities). A 13-day period observed in the velocity of the 'stationary' lines from this object⁶ shows that the system is binary. However, other models exist which also account for the

time varying optical spectrum. For example, one model suggests that the Doppler-shifted emission lines originate in a ring of material orbiting a solitary massive black hole⁷. We now report the detection of large-scale X-ray 'jets' from SS433. The X-ray emission is diffuse, extending at least 30 arc min from SS433, and is exactly aligned with both SS433 and the bulges of the shell of the huge supernova remnant (SNR) W50. This detection now (1) directly confirms the existence of jets related to SS433, (2) shows the link between SS433 and W50, proving that SS433 is galactic, (3) establishes a minimum age of the jet phenomenon of $\sim 10^3$ yr, and (4) offers an explanation of why W50 is so much larger than any other known SNR in at least one dimension. The morphology of the diffuse X-ray emission is strikingly similar to that of radio lobes of many extragalactic sources; either *in situ* heating or particle acceleration is required to explain the appearance of the diffuse X-ray emission.

The SS433 observations were made with the Einstein X-ray Observatory⁸ using the high resolution imager (HRI) in April 1979, and the imaging proportional counter (IPC) during the period 5–22 October 1979. The HRI observations show that the compact central source is unresolved (≤ 3 arc s) and has position ($\pm 5''$) of RA(1950) = 19 h 09 min 21.35 s, dec(1950) = $+04^\circ 53' 54.9''$. This position is coincident with the optical and radio position of SS433 (ref. 9). The IPC observations were made in a series of 2,000-s observations so as to monitor variable X-ray and radio emission. Two simultaneous X-ray and radio flares were observed and will be described elsewhere¹⁰. Figure 1 shows the sum of all these short observations for the energy range 0.5–3 keV and with a total integration time of 17,000 s.

The morphology of the diffuse X-ray emission reveals several important points. To show the diffuse features in the 1° field more clearly, the data in Fig. 1 have been smoothed giving the contours shown in Fig. 2, which is somewhat distorted by the vignetting correction applied. The two diffuse features are aligned with the central source. A straight line passing through the centres of both diffuse features and the central source goes through position angle 98° —nearly identical to the position angle of the bulges of the shell of W50 (ref. 11) and also closely aligned with the position angle of extended small-scale (arc s) radio emission^{12–14}. Note that diffuse emission, including the radio features observed by Geldzahler *et al.*¹¹, is not detected elsewhere inside the remnant, nor from that portion of the shell which crosses the upper north-west portion of our field.

The detection of the extended X-ray emission thus directly confirms for the first time that diametrically opposed jets originate from SS433 and travel nearly a degree across the sky to the eastern and western extremities of W50 where they collide with the SNR shell and cause the observed bulges. Clearly, there is significant interaction along the way between the jets and the SNR W50. The validity of the 'beambag' hypothesis for the morphology of W50¹⁵ is now confirmed. The X-ray data are quite consistent with 'beam models' involving twin processing jets and the ambiguity in these models between the inclination of the precessing axis and the opening half angle of the cone is removed. The X-ray morphology and the optical requirement that the angle between beam velocity and observer never be $< 60^\circ$ fix the cone half angle at 20° and the inclination at 80° . If the beam velocity is $\sim 0.258c$ across the whole remnant, this phenomenon has continued for at least $\sim 10^3$ yr, assuming the distance is 3.7 kpc (refs 16, 17).

Examination of Figs 1 and 2 shows that the diffuse feature in the west appears to be stronger than that in the east. If real, this may be related to the asymmetries between the east and west halves of W50 as shown in the radio map of Geldzahler *et al.*¹¹ and may be due to slight long-term changes in the balance of the jets and/or different properties of the medium surrounding SS433. However, the most significant morphological feature of the observed diffuse emission is that the brightness increases outward from SS433, forming rather elongated lobes, somewhat reminiscent of the appearance of radio-emitting lobes in radio galaxies. It is not clear whether or not the diffuse emission is completely absent near the star. The angle subtended by this

emission at the position of SS433 is significantly less than the full 40° cone angle expected if the outflowing gas followed a purely ballistic trajectory. Both characteristics indicate that the flow is influenced and confined by an external medium in the SNR. The interaction with this medium by some *in situ* process is probably the cause of the excitation in the lobes. To investigate this point further, we examined the physical state of the gas responsible for the X-ray emission.

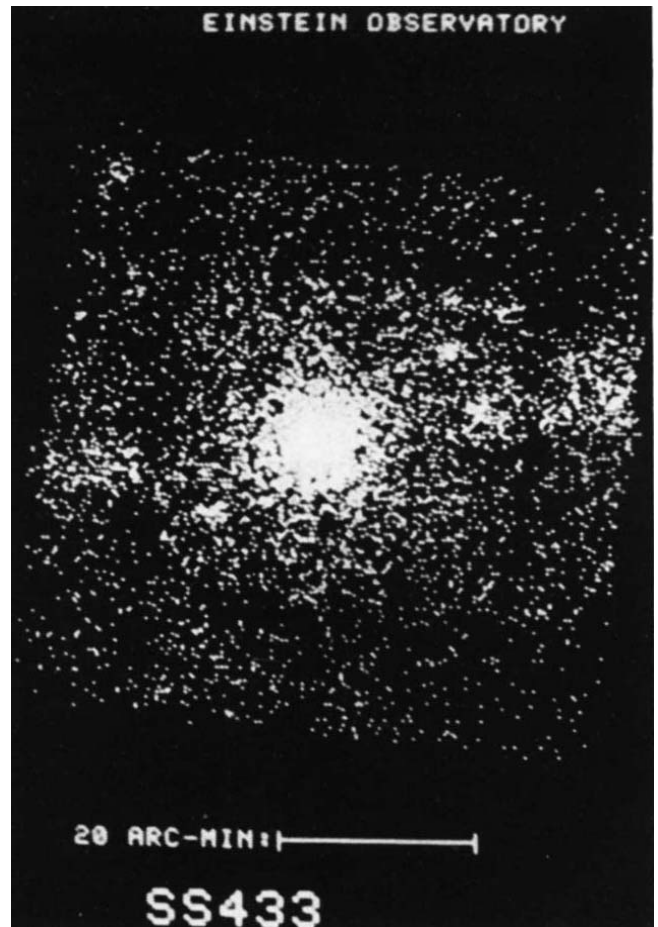


Fig. 1 X-ray emission from the central region of W50. The field of view is $1^\circ \times 1^\circ$. The bright central source is SS433. Diffuse X-ray emission is observed from a narrow region extending from east to west and roughly aligned with the central object.

The X-ray flux from the point source, SS433, is $\sim 2 \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1}$. The X-ray flux from the diffuse source is $\sim 3 \times 10^{-12} \text{ erg cm}^{-2} \text{ s}^{-1}$. At a distance of 3.7 kpc, these convert to luminosities of $\sim 10^{35}$ and $\sim 10^{34} \text{ erg s}^{-1}$, respectively. A factor of ~ 2 correction for low energy absorption (as expected for a ~ 3.7 kpc distance) has been applied. This is apparent in the X-ray spectra for both SS433 and diffuse emission, but actual spectral fits must await further IPC calibration analysis.

We consider two processes by which the beams might produce X-ray emission. First, the diffuse X rays could be produced thermally by hot plasma sustained by kinetic energy from particles in the beams. A possible mechanism for such heating is a shock generated by the beams interacting with the ambient medium (W. Tucker, personal communication). For an assumed X-ray spectral temperature of 10^7 K , the derived electron density is $\sim 10^{-1} \text{ cm}^{-3}$ and the total thermal energy contained in the plasma is $1 \times 10^{49} \text{ erg}$. If the plasma has been built up over 10^3 yr, the required energy input for heating is $3 \times 10^{38} \text{ erg s}^{-1}$, comparable in magnitude to the kinetic energy flow in the beams

computed from the spectroscopic data¹⁵. Only $3 \times 10^{-5} \text{ erg s}^{-1}$ of this energy is required to account for the energy radiated as diffuse X rays. We conclude that the beam kinetic energy is sufficient to account for this plasma, and that a significant fraction of the beam kinetic energy might be dissipated in heating the plasma.

A second possibility is that the diffuse emission, which is detected at $\sim 0.5\text{--}3 \text{ keV}$ and may thus be relatively hard, is synchrotron radiation. The *in situ* production of relativistic particles by beams in regions distinct from a central compact source is clearly suggestive of the type of processes that have been invoked for double-lobed radio galaxies and QSOs. A self-consistent synchrotron model for the X-ray emission would require a magnetic field $B \approx 10^{-5} \text{ G}$, 3×10^{42} electrons with Lorentz factors $\gamma \approx 10^8$ and energy-loss lifetimes of $\sim 10^3 \text{ yr}$. Such electrons must have a total energy content of $\sim 3 \times 10^{44} \text{ erg}$ to account for the X-ray emission. Because the electron lifetime is comparable with its expected residence time in the beam (if $v \approx 0.285c$), each relativistic electron radiates a significant fraction of its energy during the period of transit to the edge of the SNR.

As no clear radio counterpart to the X-ray beams is discernible above the radio background of the SNR, a lower limit of $\alpha \approx -0.8$ ($S \propto \nu^\alpha$) may be set for the spectral index α of the beam emission between the X-ray and radio region. Note that if $\alpha \approx -0.6$, similar to that in the radio for SS433 itself, the minimum energy in the form of relativistic electrons is 10^{47} erg , and could be as high as $3 \times 10^{48} \text{ erg}$ if the (unobservable) protons are included.

Regardless of which process is occurring, the internal co-moving energy density (or pressure) computed for the beams is within an order of magnitude of that resulting from relativistic particles and magnetic fields external to the beam and contained within W50, as known from the observed radio emission (see ref. 15). Thus, the beam may be confined laterally by the external medium within W50.

We have emphasized agreement with the kinematic jet model which is the model in best agreement with most data. Note that the X-ray data alone do not exclude other models. An accretion

disk around a black hole, for example, could supply the high velocity material observed optically and also an axis of rotation along which escaping material might flow to form opposing 'jets' responsible for the diffuse X-ray emission.

Finally, we return to the above noted similarities between SS433 and mechanisms believed to occur in radio galaxies⁵. The similarity is in morphology but is seen in different spectral regions. In SS433, X-ray emission is seen and in the radio galaxies only radio emission is detected, that is, the ratio of X-ray to radio luminosity is very different. This fundamental spectral difference could be due to the enormous difference in size scale between the phenomena of SS433 and radio galaxies, although the primary acceleration processes may be the same. Given the same initial particle energy spectrum and assuming the X rays in the SS433 beams to be synchrotron in origin, the different ratio of X-ray to radio luminosity in these objects may be due to a different ratio of radiative lifetime to residence time in the emitting volumes. In radio galaxies, the particle residence times may be sufficiently long that we now predominantly see the radiation from the lower energy particles. In SS433, however, the radiative to residence times may be comparable; hence, we see more X-ray radiation.

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Light from electron avalanches and background rejection in X-ray astronomy

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We have developed the parallel plate imaging proportional counter to register images of cosmic X-ray sources in the focal planes of X-ray telescopes^{1,2}. An important factor determining the sensitivity of such an instrument, particularly for extended source studies, is the background count rate induced in the detector by the interaction of charged particles such as cosmic rays. This background in wire anode proportional counters can be reduced by applying risetime discrimination to the charge pulse³. We report here that a modified version of the parallel plate imaging proportional counter has been constructed as shown in Fig. 1a, to investigate the application of risetime discrimination to the scintillation pulses caused by the electron avalanche process⁴. We show that efficient background event rejection ($>90\%$) is achieved and discuss an application of this system for X-ray astronomy.

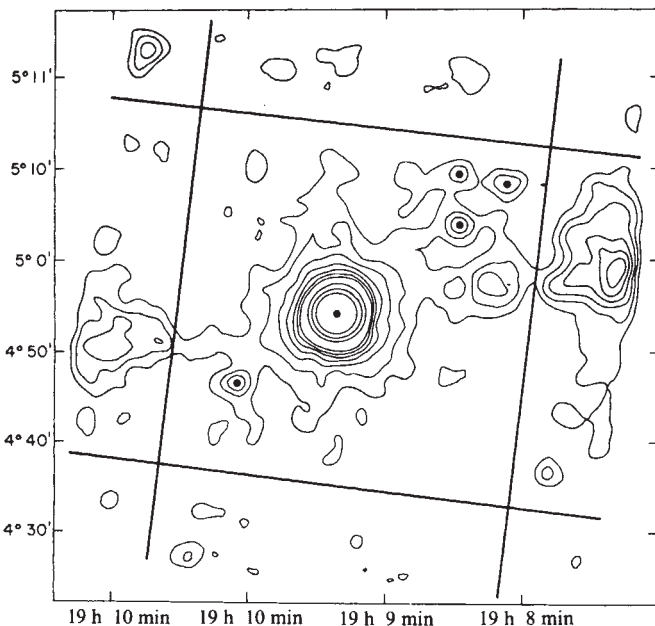


Fig. 2 Contours of constant X-ray brightness derived from data shown in Fig. 1. A correction has been made for vignetting in the telescope. Bars show detector window supports which shadow the diffuse emission. The positions of four other unidentified sources in the field are indicated. Contours showing diffuse emission are linear in increments of $5 \times 10^{-15} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ arc min}^{-2}$. Contours close to SS433 have larger increments and only show the instrument response to a strong point source.

An X-ray absorbed in the region between grid and the thin window produces a localized ionization cloud. The electrons in this cloud drift to the grid under the influence of a small electric field. A high electric field exists between the grid and the anode, accelerating the electrons to energies at which a Townsend avalanche occurs⁵. Light is emitted as a result of the ionizations and excitations which take place in this avalanche.

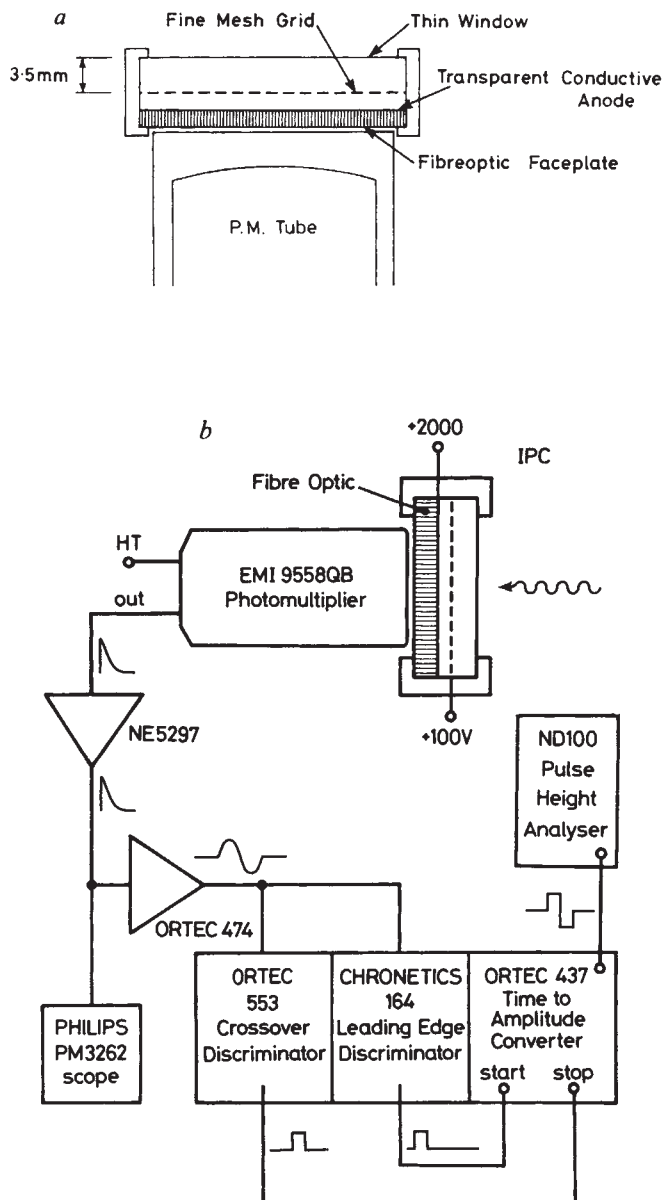


Fig. 1 a, Imaging scintillation proportional counter. b, Experimental configuration.

Figure 1b shows the experimental configuration used to investigate the risetimes of light pulses induced by X-ray and cosmic ray events. Light pulses are detected by an EMI 9558QB photomultiplier, which has an S20 photocathode and a minimum risetime of ~ 15 ns. The signals from the photomultiplier are then amplified and shaped to give a bipolar pulse. A measure of risetime is now obtained from the time interval Δt between the leading edge and crossover of the bipolar pulse. The risetime spectrum in terms of Δt was then calibrated against true risetime

by direct observation of the photomultiplier signals by a fast oscilloscope (~ 3 ns minimum response).

The risetime spectra in Fig. 2 were obtained for Al K X rays (1.487 keV) and ^{60}Co γ rays over a limited pulse height band (1.2–1.8 keV). ^{60}Co was used because it provides a reasonable simulation of 'in-flight' background for risetime discrimination systems⁹. Note that the events with risetimes faster than ~ 10 ns are due to noise.

For the gases used in this study the size of the electron cloud on reaching the grid is dominated by diffusion². For Ar (90%)/CH₄ (10%) the maximum size (FWHM) of the electron cloud produced by an X-ray photon of a few keV is ~ 0.7 mm (ref. 6) (for a drift region 3.5 mm deep and a drift field of 300 V cm^{-1}). If the time development of the scintillation pulse is determined by the drift of the electron cloud across the grid, an estimate of the pulse risetime may be obtained by dividing the cloud size by the drift velocity. In Ar (90%)/CH₄ (10%) the drift velocity for the field of 300 V cm^{-1} is $\sim 5 \text{ cm } \mu\text{s}^{-1}$ (refs 6–8), giving a maximum pulse risetime of 14 ns. As this is comparable with the 15 ns minimum time response of the photomultiplier all the X-ray risetimes are centred on this value (Fig. 2). More recent investigations with a faster photomultiplier (EMI 9818 QBM, ~ 2 ns minimum response) have shown that the maximum risetime is ≤ 14 ns.

Relativistic charged particles such as cosmic rays or Compton electrons resulting from γ -ray interactions produce an extended track of low specific ionization in the drift region. Such particles may, therefore, give rise to energy pulses in the range of the X rays to be detected and will consequently contribute to the background. The scintillation pulses from these events will have risetimes up to a maximum given approximately by the total drift depth divided by the drift velocity. For a drift space of 3.5 mm and a drift velocity of $5 \text{ cm } \mu\text{s}^{-1}$, a maximum pulse risetime of ~ 70 ns results (Fig. 2). Both risetime distributions in Fig. 2 are consistent with the above estimates.

Figure 2 shows that charged particle rejection may be achieved by using risetime discrimination on the light pulse in a similar manner to that used for the charge pulse in proportional counters. Figure 3 demonstrates the ^{60}Co γ -ray rejection efficiency as a function of the X-ray rejection efficiency. It is possible to reject 91% of the γ -ray events for a loss of only 5% of the X-ray events. This represents a significant improvement over present parallel plate proportional counters² in which only $\sim 50\%$ of the γ -ray events may be rejected using charge pulse risetime discrimination. For X-ray astronomy this will result in a significant increase in instrument sensitivity. The sensitivity of an X-ray telescope of a fixed area is determined by the diffuse X-ray background, the cosmic ray background and the spatial resolution of the instrument (in the case of point sources). Rocket flight experience with a similar detector allows us to

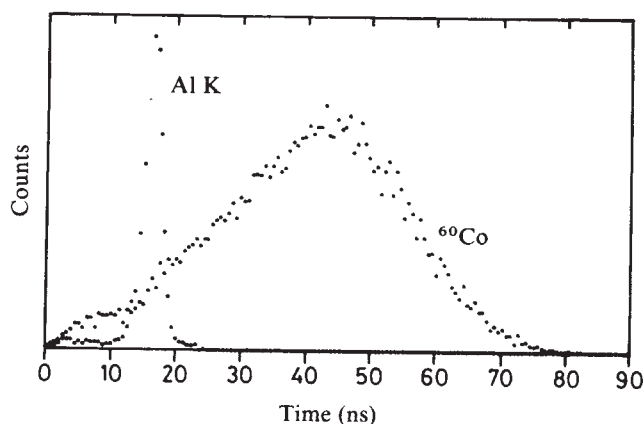


Fig. 2 Risetime spectra for a Al K X rays and ^{60}Co γ rays.

estimate the cosmic-ray background expected in flight. Taking an effective mirror area of 500 cm^2 for the range $0.1\text{--}2 \text{ keV}$ we find that the count rate per pixel from the diffuse X-ray background is $\sim 50\%$ of the cosmic ray rate at low magnetic latitudes. Hence rejection of 90% of the cosmic ray background will allow the telescope sensitivity to be determined by the diffuse X-ray background, and independent of latitude variations in cosmic ray background, which can be as much as a factor of 3. This is especially important for studies of extended sources because the signal-to-background ratio is independent of the detector pixel size.

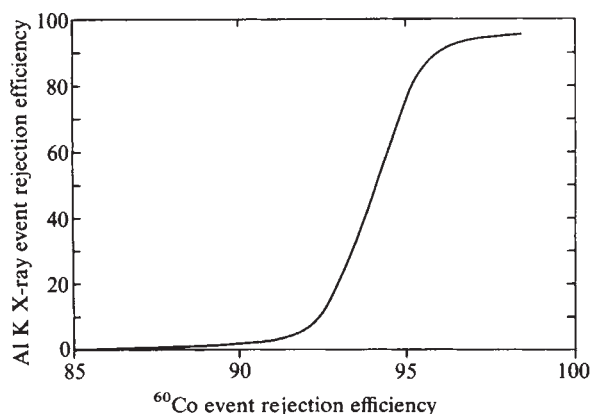


Fig. 3 ^{60}Co background rejection efficiency for risetime discrimination.

Note that for a geometrical X-ray telescope mirror area of $1,000 \text{ cm}^2$, a third magnitude star would result in 2×10^5 individual photon events s^{-1} in the photomultiplier, after attenuation by the counter window and anode. Most of these events will not be registered if a discriminator is used with a threshold set at a few photoelectrons equivalent pulse height. The background light from stars should not generally present a problem, however, because the density of stars brighter than third magnitude is only $2.5 \times 10^{-3} \text{ deg}^{-2}$.

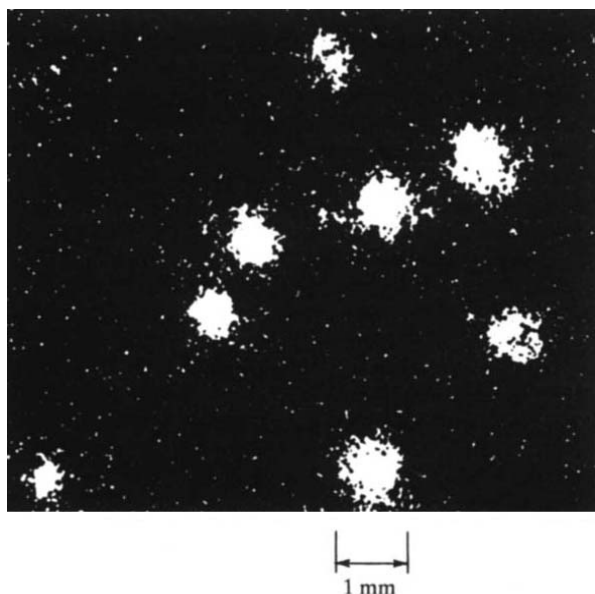


Fig. 4 Scintillation flashes produced by individual ^{55}Fe X rays in $\text{Ar (90\%)-CH}_4 \text{ (10\%)}$.

An investigation of the spectral distribution characteristics of the light emitted by $\text{Ar (90\%)/CH}_4 \text{ (10\%)}$ has shown that there are two main emission bands centred on 750 nm and 420 nm , both bands having FWHM of $\leq 50 \text{ nm}$. The ratio of the intensity of the band at 750 nm to that at 420 nm is about $14:1$. At a charge gain of 5×10^4 in $\text{Ar (90\%)/CH}_4 \text{ (10\%)}$ using Al K X rays a total of the order of 10^4 photons are produced for each X-ray photon event. Because the main emission band at 750 nm is reasonably narrow it should be possible to reduce the starlight background by factor of 15 using a narrow band filter transmitting between 700 and 800 nm .

Because most of the scintillation emission occurs within the last mean free path of the electrons in the avalanche region, it is possible to locate individual X-ray events from the scintillation pulse using a fibre optic faceplate and a transparent anode deposited on its surface. Figure 4 shows the localized flashes produced with $\text{Ar (90\%)/CH}_4 \text{ (10\%)}$ under irradiation by $\text{Mn K X rays (5.9 keV)}$, recorded on film by means of an image intensifier. We are developing an optical detection system which will enable the centroid of each flash to be determined electronically. This work should lead to the development of a detector whose positional resolution is limited only by diffusion of the primary electron cloud in the drift space.

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Distribution of cosmic-ray electrons in the Galaxy

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Although it seems that cosmic-ray electrons in the range $100\text{--}300 \text{ MeV}$ are mostly generated in sources within the Galaxy (extragalactic electrons are kept out by the 2.7 K relic radiation) their distribution is not known. Studies of electron synchrotron radiation in the region of hundreds of MHz indicate that the synchrotron emissivity varies with position, generally decreasing as the galacto-centric distance, R , increases, but such dependence could be in either the electron flux, I_e , or the magnetic field strength, H , (emissivity $\propto I_e H^2$) or both. We examine here the distribution of I_e itself from studies of the γ -ray flux produced by electrons interacting in the interstellar medium by way of the dominant bremsstrahlung mechanism.

Using γ -ray data to find the distribution of cosmic rays has long been considered; for example, Dodds *et al.*¹ have reviewed the distribution of a mixed proton-electron component from analysis of γ rays above 100 MeV towards the galactic anti-centre. Here, SAS 2 satellite data²⁻⁴ is used in the energy range 35–100 MeV coupled with information on the distribution of target gas in the Galaxy.

In this energy range, the 1σ radius of the angular resolution function of the SAS 2 telescope for individual photons is 6° . Fichtel *et al.*² presented their data in the form of a number of photons observed in bins of equal solid angle (6.06×10^{-4} sr), each covering 2.5° in longitude, together with the relative sensitivity of the telescope for each bin. The above energy range is useful because the contribution to the γ -ray intensity from protons and heavier nuclei is small. Proton-induced γ rays come mainly from π^0 decay and the resulting γ spectrum has a peak at about 70 MeV ($m_{\pi^0}c^2/2$) whereas the spectrum of γ rays from electron bremsstrahlung falls continuously with increasing γ -ray energy. The overall spectral shape of γ rays indicates that π^0 and e-production do not become equal until ~ 170 MeV, leading to the conclusion that only $\sim 10\%$ of cosmic γ rays in the energy range 35–100 MeV result from π^0 decay. For γ rays in this energy range the corresponding electron energy is between about 70 and 300 MeV.

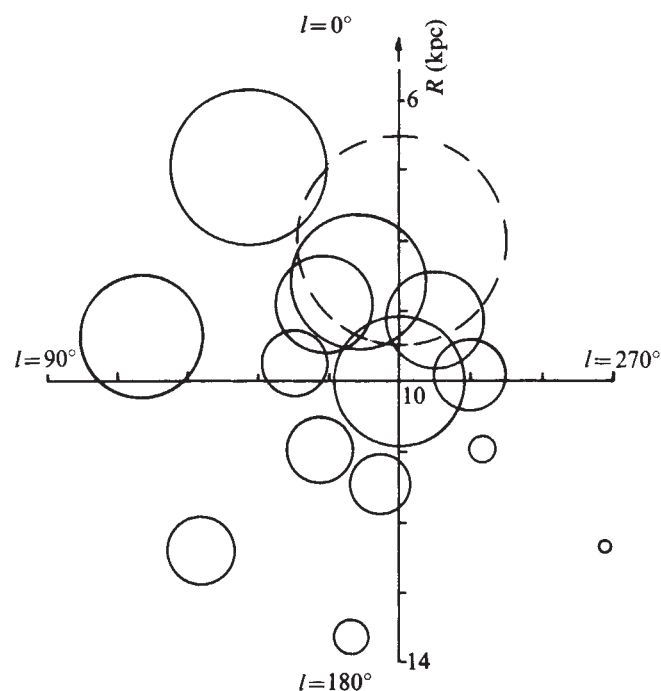


Fig. 1 Plan view of the Galaxy showing the intensity of cosmic-ray electrons in the region of several hundred MeV as a function of position in the local region. The electron intensity is proportional to the radius of the circle. Uncertainties in intensity are typically 30%. For absolute intensities see Fig. 2. The centres of the circles are the average positions to which the intensities refer; the positions are necessarily imprecise (uncertainty $\approx 30\%$ of distance to the Sun). The dotted circle relates to an attempt to allow for the contribution of discrete sources; the uncertainty is greater than for the other intensity estimates. The vertical scale is the distance from the galactic centre, R .

Previous analyses⁵⁻⁷ have studied facets of this problem (although not in the present energy range) but here we examine the problem using recent data on gas densities and taking full recognition of various experimental factors (for example, the angular resolution function of the telescope). The object is to answer the specific question: how are energetic electrons distributed in the Galaxy?

We divide the γ -ray data into bands of longitude and latitude and for each find the value of $q/4\pi$ in the expression $I_\gamma = (q/4\pi)N_H + I_b$, where N_H is the volume density of target gas and I_b is the background (or more specifically, the component uncorrelated with the gas). q is proportional to the average electron intensity along the appropriate line of sight and, as a first approximation, can be taken to be proportional to $I_e(l, b, r)$ where r is the distance along the line of sight to a point $z(\frac{1}{2})$ above the galactic plane, $z(\frac{1}{2})$ being the half-thickness of the gas layer.

We have not tried to remove the contribution of 'discrete' sources from the data because we believe that many are due to molecular clouds irradiated by the ambient cosmic ray flux. As the sources CG184-5 and CG263-2 have been unambiguously identified with the Crab and Vela pulsars, however, data from within a few degrees of these sources are not used in this analysis.

This approach has several problems, the most important being that, of the gas constituents, only the atomic column densities are available for most of the range of l and b (here we adopt the Berkeley measurements⁸). The important H_2 column densities are readily available in restricted regions only, for example, $10^\circ < l < 82^\circ$, $b = 0^\circ (\pm 1^\circ)$ (refs 9, 16) and from indirect analyses using galaxy counts¹⁰⁻¹² at $|b| > 10^\circ$. The low counting statistics involved in the γ -ray results mean that a large area of the sky must be considered to obtain results with sufficient accuracy to be useful and such a wide coverage is not yet available in the case of the H_2 column densities. The H_2 column densities are, nevertheless, too large to be ignored completely. Gordon and Burton⁹ presented measurements of the 2.6-mm line of CO which suggest that locally the surface densities of H I and H_2 (in atoms cm^{-2}) are about the same but their data for the local region (within 2 kpc of the Sun) may be too high, for two reasons. First, the quoted value for the local region is an average round the Galaxy at $R \sim 10$ kpc and most of the weight for the actual value comes from regions very remote from the Sun. The second point is that the formaldehyde results¹³, Copernicus UV measurements¹⁴, the analysis of Blitz and Shu¹⁵ and our Galaxy count results favour values of N_{H_2}/N_{H_I} in the range 0.25–0.5. The lack of detailed knowledge of the H_2 column density in the directions being studied can, however, be at least partially overcome if the correlation between H I and H_2 column densities can be shown to be small.

General arguments suggest poor correlation, mainly the fact that the molecular hydrogen occurs largely in dense dumps and the well-known observation that the thickness of the H_2 layer is only half that of the atomic hydrogen layer. A more detailed analysis gives the following. Galaxy count studies show a negligible correlation for the range $60^\circ < l < 240^\circ$ but a significant correlation for $10^\circ < l < 60^\circ$ (all for $|b| = 10\text{--}20^\circ$). This negligible correlation for $60^\circ < l < 240^\circ$ would also be expected to apply for latitudes below 10° , largely because in the outer Galaxy the amount of H_2 decreases rapidly with increasing galactocentric radius. Thus, we believe that the q -values derived from an I_γ , N_{H_I} analysis give a good representation of the trend of I_e and R here, that is for $R \geq 9$ kpc (we assume that the sum is at $R = 10$ kpc).

In the inner Galaxy the correlation between H I and H_2 causes more difficulty, but even here it is not insurmountable. Inspection of the CO and H I data at $b = 0^\circ$ (summarized by Burton and Gordon¹⁶) show that both follow the same general decrease over the range $l = 10\text{--}65^\circ$. However, large variations in the column densities of both components also occur on a smaller scale ($\Delta l \sim 1\text{--}5^\circ$). These smaller-scale variations are sometimes of the order of 50% of the overall decrease in the range $l = 10\text{--}65^\circ$ and the variations in CO seem uncorrelated with those in H I. If the longitude range is split into the two ranges $l = 10\text{--}35^\circ$ and $l = 35\text{--}65^\circ$, to be considered separately, then the smaller-scale variations are found to be large enough compared with the overall variation in column density to treat the H I and H_2 column densities as being effectively uncorrelated. The limited statistical accuracy of the data and the fact that the angular resolution is several degrees means that a rather large range of

latitude must be considered (we take various regions, mainly with $|b| < 5.6^\circ$ and $|b| = 2.4\text{--}12.8^\circ$); thus, the contribution of H_2 will be much smaller than that at $b = 0^\circ$ and the correlation effect even smaller. Considering $|b| < 12.8^\circ$ the values of $(q/4\pi)$ are 3.4 ($l = 10\text{--}65^\circ$), 3.8 ($l = 10\text{--}35^\circ$), and 2.8 ($l = 35\text{--}65^\circ$) $\times 10^{-26} \text{ s}^{-1} \text{ sr}^{-1} \text{ atom}^{-1}$. These indicate that there is very little correlation in this range, as correlation would cause the q value for $l = 10\text{--}65^\circ$ to be bigger than for the divided ranges.

The data are analysed by plotting I_γ against $N_{H\text{I}}$ (from ref. 8) and finding the maximum likelihood estimators of $q/4\pi$ and I_b in the expression $I_\gamma = (q/4\pi)N_{H\text{I}} + I_b$. If $N_{H\text{I}}$ is uncorrelated with $N_{H\text{I}}$ then the mean contribution of H_2 to the γ -ray flux in the region being considered will appear as a component of I_b , leaving $q/4\pi$ as the γ -ray emissivity of the atomic hydrogen gas. The present analysis differs from that of Fichtel *et al.*⁶ in including the angular resolution of the SAS 2 telescope and in the more detailed analysis of the data in each longitude and latitude range.

Figure 1 shows the relative values of I_e ; the radii of the circles are proportional to $q/4\pi$ at the position in the galactic plane (relative to the Sun) represented by their centres. The distance from the Sun to which each measurement of $q/4\pi$ refers is taken to be the distance along the line of sight to a point $z(\frac{1}{2})$ above the galactic plane. The goodness of fit of the lines to the data points gives an estimate of the uncertainty in the value of $q/4\pi$ and this is roughly 30%. Figure 1 shows a dispersion of intensities about a smooth dependence on galacto-centric radius such as corresponds to the estimated individual errors except for the intensities in the quadrant $l = 180^\circ$ to 270° are singularly low. Such very intensities are probably real, however, and no doubt relate to the dearth in likely cosmic ray sources in this region (such as H II regions, and OB associations). The low values in general in the direction of the anticentre are particularly firm in view of their lack of contamination by the effects of molecular hydrogen.

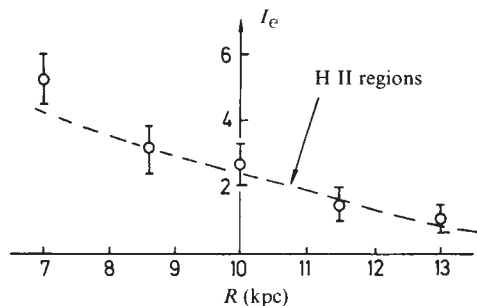


Fig. 2 Average intensity of cosmic-ray electrons as a function of galacto-centric radius, R , for the region covered by Fig. 1. I_e ($R = 10 \text{ kpc}$) $\approx 8 \times 10^3 \text{ m}^{-2} \text{ s}^{-1} \text{ GeV}^{-1}$. The dotted line is the average distribution of the number density of H II regions above a fixed threshold¹⁵. H II regions are not necessarily the sources of the electrons: many other astronomical objects have similar radial distributions, although it is interesting that the dearth of electrons at $R < 2 \text{ kpc}$ is mirrored by a deficit of H II regions (this is a difficult region in which to see the other objects) and the same situation arises for the region $l, 180^\circ\text{--}270^\circ$.

The data can be combined, assuming radial symmetry, to give the average radial distribution of electron intensity in the vicinity of the Solar System (within a few kpc of the Sun). Figure 2 shows the result.

The distribution of electrons nearer to the galactic centre than shown in Figs 1 and 2 can be studied using the unfolding

technique to give the radial distribution of γ -ray emissivity; this is then divided by the gas density to give the electron intensity. Such unfoldings of the γ -ray data have been performed^{17,18} and assume cylindrical symmetry of the γ -ray emissivity of the galactic disk to derive its radial distribution from the variation of the flux with galactic longitude. The method suffers because of large uncertainties in the amount of molecular gas in the inner Galaxy. Here, as no sophisticated analysis is possible, the earlier results are very doubtful.

New data on gas densities^{19,20} have renewed interest in this method, however, at least for the central region of the Galaxy ($R \leq 2 \text{ kpc}$). These data give a much higher mass of gas than previously suspected (and account for its non-observation previously). Specifically, Liszt and Burton¹⁹ quote a mass of at least $10^9 M_\odot$ and Linke *et al.*²⁰ favour $8 \times 10^9 M_\odot$. These masses are high enough to derive important conclusions even allowing for some uncertainty in their magnitude.

We have made a new determination of the emissivity versus galacto-centric radius using the γ -ray line flux from Fichtel *et al.*⁴ in the range 35–100 MeV. The γ -ray emissivity averaged within $R = 2 \text{ kpc}$ is $\sim 10^{40} \text{ } \gamma\text{'s (35--100 MeV) kpc}^{-2} \text{ s}^{-1}$ (an essentially upper limit which does not depend at all sensitively on the unfolding assumptions), and the electron intensity required, using a mass of $8 \times 10^9 M_\odot$, is only 4% of the local value. Such a small value is difficult to explain in terms of exclusion of electrons from molecular clouds insofar as the gas seems to be rather smoothly distributed¹⁷ rather than in clumps which could conceivably have associated excluding magnetic fields. If the gas data are correct it suggests that the electron intensity is very low (the arguments here relate to a region much larger than the 300-pc ring very close to the galactic centre for which different arguments apply²¹). Evidence on the deficit of likely sources of energetic electrons at $R < 2 \text{ kpc}$ is hard to come by; however, some data on H II regions (which are indicators of energetic phenomena) show a similar deficiency below 2 kpc (ref. 20). The most important feature is probably that not only are sources infrequent here but that diffusive motion is so small and the space is not filled with particles propagating from elsewhere.

Clearly there are significant variations in the intensity of cosmic-ray electrons over the Galaxy. A fall off at $R > 10 \text{ kpc}$ and a reduction by at least an order of magnitude, compared with the local intensity, for $R < 2 \text{ kpc}$ seem particularly firm.

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The dynamic expansion and contraction of the jovian plasma sheet

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We report here that near noon in the magnetosphere of Jupiter, plasma is observed to flow away from the equatorial current sheet. The motion reverses near dusk, with a subsequent infall of plasma towards the sheet on the nightside. We associate this motion with the compression of magnetic flux tubes on the dayside and their subsequent expansion on the nightside due to the solar wind interaction with Jupiter. Such compression and expansion may be sufficiently rapid that the plasma in the sheet cannot reach quasi-static equilibrium. Dynamic motions at transonic velocities ensue.

The plasma instrument on board the Voyager spacecraft consists of four modulated-grid Faraday cups which measure positive ions and electrons with energies per charge in the range 10–5,950 V (ref. 1). Three of these cups are arranged in a cluster whose symmetry axis normally points towards the Earth. The fourth cup (the D cup) is situated so that its axis is perpendicular to the symmetry axis of the main cluster. The trajectory of Voyager 2, and the orientation of the sensor normals at various points along that trajectory, are shown in Fig. 1; the Voyager 1 trajectory and sensor orientations are qualitatively similar. Plasma flow in the jovian magnetosphere has a tendency to co-rotate with the planet, that is, to move azimuthally at the rotation rate of the planet. The D cup is orientated so that azimuthal flow moves almost directly into that cup on the inbound leg of the trajectory. Near closest approach, strictly co-rotating flow would move into the field of view of the main sensors, and after closest approach out of the field of view of all sensors.

The response functions² of the Voyager Faraday cups are broad, with 30% response at an angle of incidence of 60° to axes of cups in the main cluster, and 30% response at an angle of 40° to the side sensor axis. Over much of the inbound trajectory, there is at least one sensor for which the plasma flow velocity is within ~20° of the sensor normal. If, in addition, the plasma is reasonably supersonic ($Mach \geq 3$), the response function can be taken as unity. In such a situation, the analysis of the positive ion data from that sensor is relatively straightforward, although complicated by the fact that the jovian magnetospheric plasma consists of an admixture of many ionic species (H^+ , S^+ , S^{2+} , S^{3+} , O^+ , O^{2+} , Na^+ , SO_2^+). Such single sensor data have been exhaustively analysed since the Jupiter encounters for heavy ion densities and temperatures, as well as for the velocity component along the sensor axis^{3–5}. However, only for a brief time near closest approach, and during isolated spacecraft manoeuvres, is the unit response assumption valid for more than one sensor. To obtain all three components of the plasma velocity, we must use information from at least three sensors; because two of the three are usually significantly oblique to the flow, it is necessary to use the full response functions of the sensors. The complete determination of the velocity is more complex and time consuming, and at present can only be done on a limited basis. We discuss some quantitative results from this analysis below.

First, however, we take advantage of the symmetry properties of two of the main cluster sensors with respect to the jovian equatorial plane to obtain some qualitative results about the plasma velocity component perpendicular to that plane. These

qualitative results are quantitatively verified by our more sophisticated analysis below. When the attitude of the spacecraft is referenced to the star Canopus, the axes of the A and D cups are essentially in the jovian equatorial plane. The axes of the B and C cups, however, are inclined to that plane in a nearly symmetrical fashion, at angles of 18.6° south and 15.3° north, respectively, for Voyager 2. We compute the flux density in a given cup by taking the total current due to positive ions with energies per charge from 10 to 5,950 V, and dividing by the proton charge and the effective area of the sensor. Figure 2 shows the flux density of the B cup minus the flux density of the C cup, divided by the sum of the flux densities in the B and C cups for a portion of the Voyager 2 trajectory. Because of the orientation of the cups, this difference will be positive for flow towards the north and negative for flow towards the south. Figure 2 also plots the distance of the spacecraft above the magnetic dipole equatorial plane. We indicate at various points the local time, and the distance of the spacecraft from Jupiter, with periapse at 10.1 jovian radii (R_J) (9 July 1979 at 2230 h). We have only plotted data in Fig. 2 when the fluxes in the B and C sensors are high enough to allow a significant comparison, and when the spacecraft was on Canopus. Unfortunately, this means that we can use little data past 1400 h on 10 July 1979.

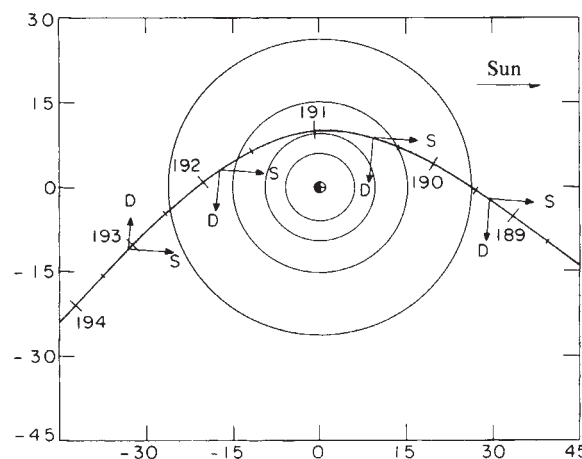


Fig. 1 The Voyager 2 trajectory projected onto the jovian equatorial plane. The line labelled S is the projection of the symmetry axis of the main cluster of Faraday cups, and the line labelled D is the projection of the D cup axis. The A and D cup axes are essentially in the jovian equatorial plane, with the A cup axis ~110° from the D cup axis. The B and C cup axes are inclined ~17° south and north of that plane, respectively, ~75° from the D cup axis. Tic marks along the trajectory are shown every 12 h, with day of year at the beginning of the day (day 190 is 9 July, 1979). The orbits of the four Galilean satellites are also shown. Distances are given in R_J .

Crossings of the magnetic dipole plane are reasonably accurate markers for crossings of the jovian current sheet, especially inside $20R_J$. From the Voyager 2 data in Fig. 2, and from similar plots of Voyager 1 data, it is clear that in the dayside jovian magnetosphere near noon, there is a marked tendency for plasma flow to be northward when the spacecraft is north of the magnetic dipole plane, and southward when the spacecraft is south of that plane—thus, the flow is away from the current sheet. This pattern is the same as that observed in Pioneer measurements of the anisotropies of MeV protons in the dayside middle magnetosphere^{6,7}. The amplitude of the B–C difference decreases to zero with decreasing radius and increasing local time towards dusk. In the dusk to midnight sector, the B–C

difference again increases with increasing radius and increasing local time towards midnight. However, in this sector the plasma flow tends to be northward when the spacecraft is south of the magnetic equator, and southward when it is north of the equator—that is, the flow is now towards the current sheet.

The flow pattern can also be seen in a detailed inspection of the positive ion spectra from the various sensors. Figure 3 shows a low-resolution scan of the positive ion flux density near the crossing of the magnetic equatorial plane near $20R_J$ on Voyager 2 inbound (see Fig. 2). The flux density spectrum in the D sensor is an unresolved superposition of H^+ , O^{2+} , S^{3+} and S^{2+} (refs 4, 5). At this time, the B and C sensor normals are $\sim 73^\circ$ and $\sim 67^\circ$ from the rigid co-rotational flow velocity, with the D sensor normal only $\sim 12^\circ$ from that velocity. From these spectra, we conclude qualitatively that the flow velocity is mostly azimuthal at this time because the D sensor flux is much higher than fluxes in the main sensor. Moreover, the flow velocity must lie near the equatorial plane because of the near equality of the fluxes in the B and C cups. In contrast, Fig. 4 shows a low resolution scan of the positive ions during a dayside excursion of the spacecraft to $\sim 3.3R_J$ below the magnetic equatorial plane. The B and C sensor fluxes are comparable with those in the D sensor even though the sensor orientation is qualitatively the same as for the spectra in Fig. 3. This implies a significant component of velocity radially towards the planet during this interval. In addition, the C cup flux is larger than the B cup flux, implying a southward

tially done in stages using an integration code which includes the full sensor responses. For example, we quote results obtained by Voyager 2 near 2009 h on 8 July 1979 (Fig. 4). The spacecraft was near noon, $21.6R_J$ from Jupiter and $-3.3R_J$ below the magnetic dipole plane. An analysis of the proton peak in the B, C and D sensors yields a density of 0.12 cm^{-3} and a thermal speed of 120 km s^{-1} . In a cylindrical coordinate system, where the z -axis is the rotation axis of Jupiter, the radial component of the proton velocity was -167 km s^{-1} , the azimuthal component was 264 km s^{-1} and the z -component was -95 km s^{-1} . These non-azimuthal velocity components should not be taken as typical, since for quantitative analysis, we must at present choose the most pronounced examples of the phenomenon. The azimuthal component here is slightly less than the 270 km s^{-1} velocity expected for rigid co-rotation³. In this interval, the plasma was flowing southward (away from the current sheet), in agreement with Fig. 2, and was also flowing radially towards Jupiter, a feature which cannot be deduced from Fig. 2. An analysis of spectra near midnight (at 1300 h on 10 July, 1979) yields similar results, except that the flow is now northward (towards the current sheet) and away from Jupiter.

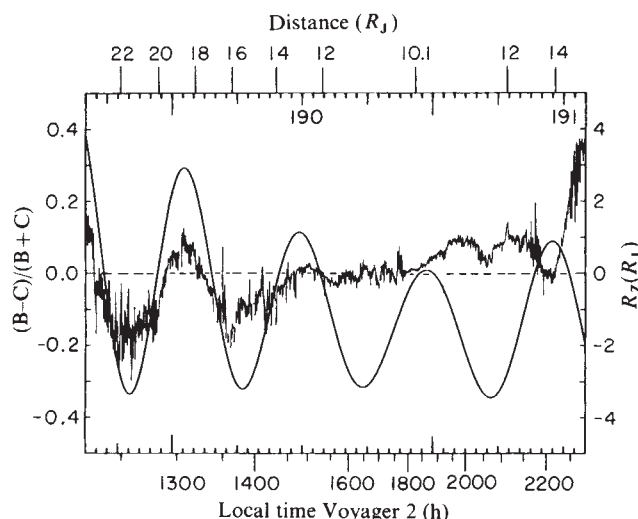


Fig. 2 The difference between the B and C cup fluxes divided by the sum of those fluxes, as a function of time during the Voyager 2 encounter, from 1600 h on 8 July, 1979 (day 189) to 1400 h on 10 July, 1979 (day 191). A positive value for this difference indicates northward flow. Also shown is the distance of the spacecraft above a magnetic dipole plane. Interior tick marks give spacecraft time at one hour intervals, with day of year given at 1200 h. We also indicate values of the local time and the radial distance from Jupiter.

component of flow and thus a flow away from the current sheet. We also note that the main sensor is orientated such that there would be slightly more purely azimuthal flow into the C cup than the B cup on the inbound leg, and vice versa on the outbound leg. Thus even in the absence of any north/south flow, we qualitatively expect to see a negative signature in $(B-C)/(B+C)$ inbound and a positive signature outbound. In fact, the differences in Fig. 2 oscillate about a negative average value inbound and a positive average value outbound, as expected.

This qualitative information on flow patterns is borne out by quantitative analysis of detailed measurements of the positive ion distributions in the various sensors. This analysis is essen-

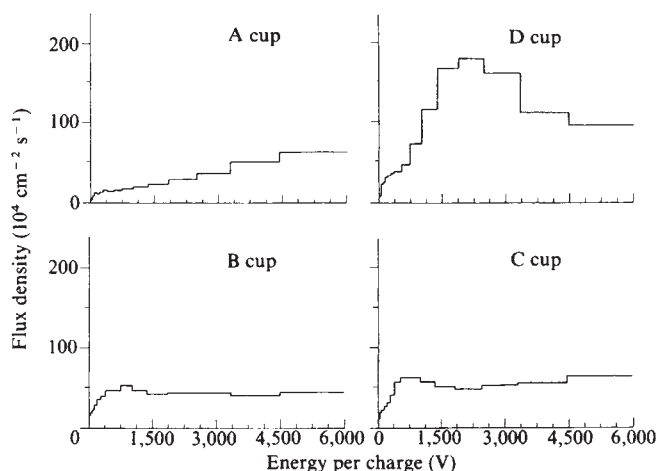


Fig. 3 A low resolution scan of the flux density of positive ions from Voyager 2 inbound $20.0R_J$ on day 189 of 1979 at 2250 h 47 min 54 s.

One possible interpretation of these flow patterns is as follows. In a steady state, the distribution of plasma along a given magnetic flux tube is roughly determined by the balance of the pressure gradient of the plasma and the component of centrifugal force along the field. The scale height H for a density distributed in this way is the product of the sound speed C_s , the rotation period of the planet⁸ and dimensionless factors of the order of $1/2\pi$. If this static situation is perturbed we still expect a quasi-static density distribution as long as the perturbation time scale is long relative to the dynamic time scale $2\pi H/C_s$. If the perturbation is short relative to this time scale, the plasma cannot adjust in a quasi-static fashion, and plasma motions with velocities up to C_s and beyond can result.

The dynamic time scale for the jovian plasma sheet is thus some fraction of the order of unity of the rotation period of the planet. However, the plasma sheet is perturbed on time scales of this order due to the compression of the dayside magnetosphere by the solar wind. Plasma will tend to move away from the plasma sheet as it moves into the dayside magnetosphere, because of both the compression and the decreasing component of centrifugal force along the field. Because of the rapid time scale, the adjustment of the plasma density distribution along the field line will not be quasi-static, and bulk motion along the field and away from the sheet can ensue. This motion will lead both to a north/south flow and a flow towards the planet on the dayside, as observed. At decreasing radial distances, the

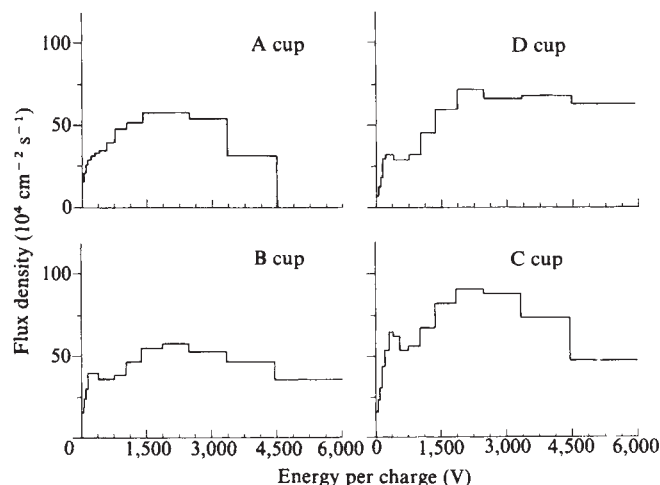


Fig. 4 A low resolution scan of the flux density of positive ions from Voyager 2 inbound 21.6 R_J on day 189 of 1979 at 2009 h 11 min 55 s.

perturbation associated with the solar wind is smaller and leads to a perturbed flow whose amplitude decreases with decreasing radial distance near noon, also in accord with observations (Fig. 2 and ref. 7). Past noon, the magnetic flux tube will begin to expand and move to larger distances. The expansion of the flux tube and the increasing component of centrifugal force will lead to a reversal of the plasma flow near dusk, and cause an infall of material at transonic (or supersonic) velocities in the nightside current sheet.

We note that this picture is very different from what would be expected from a quasi-static expansion and contraction of the plasma sheet due to the solar wind interaction. A quasi-static process would produce a sheet which was thickest at maximum compression (noon), and thinnest at minimum compression (midnight). Any small velocities associated with such a quasi-static process would be zero at noon and midnight, and maximal at dawn and dusk. In contrast, the dynamical velocity pattern described above, especially its phase, arises only in the absence of quasi-static equilibrium. The possibility of such a pattern depends on the fact that the solar wind interaction tends to perturb a given flux tube on the same time scale as it takes the plasma to come to static equilibrium.

These observations and our interpretation of them have two obvious implications for both theory and observation of the jovian magnetosphere. First, irreversible processes such as internal shocks or effective viscosities will lead to a net heating of the plasma as it expands away from and collapses towards the plasma sheet (that is, a fluid analogy of the acceleration mechanism of Goertz⁹). Second, theoretical models of the plasma sheet which assume quasi-static equilibrium, and interpretations of data based on such models, must be re-examined.

We thank H. S. Bridge and S. Olbert for discussions. This work was supported in part by NASA contract 953733.

Note added in proof: It has been suggested that the solar wind perturbation is slow enough to allow quasi-static solutions. This reasoning is based on a comparison of the solar wind perturbation time scale to the quasi-static equilibration time scale. Here we attempt to clarify our argument. In a plane parallel atmosphere,

$$\rho \frac{\partial \mathbf{v}}{\partial t} = -\nabla p - \rho \mathbf{g}$$

where $\mathbf{v}$ is the plasma velocity, ρ the mass density, p the pressure, and $\mathbf{g}$ the gravitational (real centrifugal for Jupiter)

acceleration. Taking a time derivative and using mass conservation

$$\rho \frac{\partial^2 \mathbf{v}}{\partial t^2} = +\rho C_s^2 \frac{\partial^2 \mathbf{v}}{\partial z^2} + g \rho \frac{\partial \mathbf{v}}{\partial z}$$

where we have assumed that $\mathbf{g}$ and $\mathbf{v}$ are in the z direction, and we are only keeping terms of interest for order of magnitude (we have dropped many other terms). Assuming an $\exp(i(kz - \omega t))$ dependence for $\mathbf{v}$, we have

$$\omega^2 = C_s^2 k^2 - igk = k(kC_s^2 - ig)$$

The static or quasi-static solution is obtained by setting ω^2 to zero and obtaining $k = +ig/C_s^2$ and thus an $\exp[-z/H]$ falloff, with $H = C_s^2/g$. This solution will be valid as long as $\omega^2 \ll C_s^2 k^2$, or $\omega \ll C_s H$. Thus, the angular frequency of any sinusoidal perturbation should be compared to $C_s H$ to determine if the response of the system will be quasi-static.

At Jupiter, C_s/H is the angular rotation rate of the planet ($2\pi/10$ h), to factors of the order of unity ($\sqrt{2}/3$). The angular frequency of the solar wind perturbation is ($2\pi/10$ h). The similarity between these two frequencies is the basis for our contention that the quasi-static situation may not obtain. We have been rather sloppy in this derivation, but the overall conclusion remains even in a more careful consideration.

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Io control of jovian radio emission

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The stimulation by Io of some fraction of the jovian decametre-wavelength radio emission was recognized in 1964¹. Since then many observations have indicated that strong Io control is limited to frequencies between about 22 and 40 MHz (refs 2–4). At lower frequencies, that is 8–10 MHz, the degree of Io modulation declines markedly⁵. Recent low-frequency RAE 1 satellite⁶, Voyager 1 (ref. 7), and groundbased⁸ studies have shed some doubt on the conclusion that Io control predominates only at high frequencies. Data from the Earth-orbiting RAE 1 satellite⁶ revealed a strong dependence of emission detection probability on Io orbital phase at frequencies in the range 6.5–2.2 MHz. These apparently conflicting results lead to two different conclusions: (1) Io control decreases markedly in the 10–20 MHz regime but reappears near 6 MHz, or, (2) observations above 8 MHz require⁵ that the emission intensity be taken into account. We demonstrate here that the latter is the case and offer a reinterpretation of these data which is consistent with the recent low-frequency satellite and groundbased results.

Figure 1 shows the results of a two-year survey conducted at 26.3 MHz. These observations, a preliminary analysis of which was reported earlier⁹, are unique in that they were made with an extremely sensitive antenna array consisting of 640 half-wave dipoles. Thus a large volume of very weak emission was detected for the first time. We divide the data into two approximately

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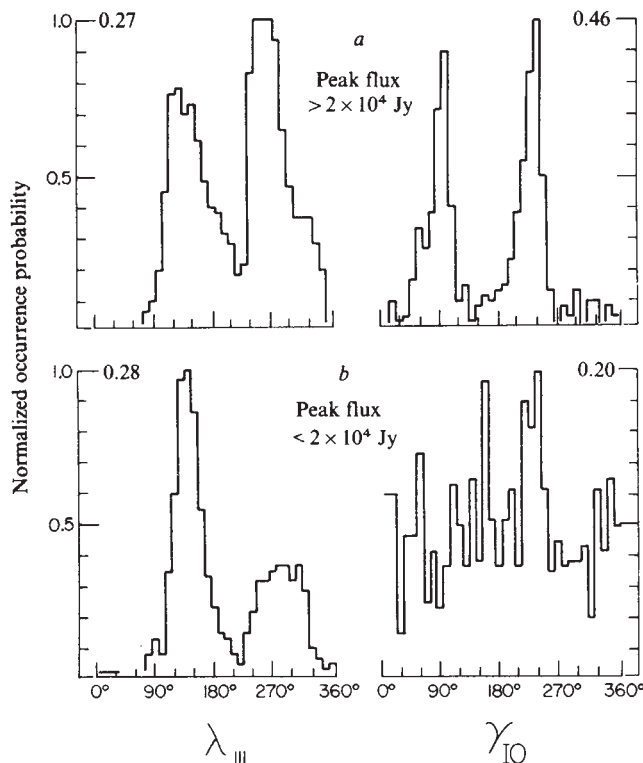


Fig. 1 A total of 151 h of 26.3-MHz jovian radio emission in which the occurrence probability is plotted as a function of the System III central meridian longitude (CML, 1965) and the geocentric phase of Io (γ_{Io}). In each case the data are divided into two components. One component (a), consisting of 83 h of activity is comprised of storms which exceeded a threshold intensity of 2×10^4 Jy (2×10^{-22} Wm $^{-2}$ Hz $^{-1}$). The weaker component (b), consisting of 68 h of activity is made up of storms which did not exceed this same level. The observing period extended from 16 April, 1973 to 1 March, 1975 and contained 997 h of interference-free monitoring. The weak emission, while displaying strong modulation in CML, exhibits virtually no control by Io.

equal parts, one consisting of radio storms whose peak intensity exceeded a certain threshold and one component which did not exceed this threshold. Both the strong and weak components are plotted as separate functions of the System III (1965) central meridian longitude (CML) on Jupiter and the geocentric phase of Io (γ_{Io}). As anticipated from previous studies, the high intensity component (a) displays strong Io control, having occurrence probability peaks at the expected Io phase locations of 90° and 240°. The weak emission (b), however, shows no preferred Io phase. Instead it exhibits random fluctuations as a function of γ_{Io} and is, therefore, considered to be Io-independent emission. This latter result was entirely unexpected because large amounts of Io-independent emission had previously been observed only at relatively low frequencies, for example at 18 MHz and below. Reliable identification of these low-level Jupiter events is assured as can be inferred from the histogram (Fig. 1b) which shows the emission plotted as a function of CML. It displays the same dependence on CML as does the high intensity component.

Thus from the above observations alone it seems that there is not necessarily a strong frequency dependence to Io control. We hypothesize that, phenomenologically at least, Io control is a flux-dependent rather than a frequency-dependent phenomenon. We test this hypothesis by performing the same analysis as above at lower frequencies, where Io control is supposed to weaken and eventually disappear.

The results of such an analysis at 18 and 10 MHz are presented in Figs 2 and 3, respectively. The plots are identical in format to Fig. 1. In agreement with the 26.3 MHz results, there is strong Io control evident at both 18 and 10 MHz when the high intensity emission is isolated. The occurrence probability peaks are centred on the classical 90° and 240° Io-phase positions; only the relative amplitudes of the peaks differ. Note also that, just as was evident at 26 MHz, the low intensity emission is independent of Io phase. This is particularly striking at 18 MHz where the statistical reliability of the data is greatest. Both data sets retain modulation in the System III coordinate at both intensity levels, the absence of which might imply significant mistaken identification of Jupiter events.

We have shown that by isolating the high intensity radio emission at 26, 18, and 10 MHz the dependence of Io control on frequency disappears to first order. The explanation for apparent Io control in previous studies is now evident if one recalls that Jupiter's flux density spectrum peaks at ~ 8 MHz and falls off steeply at higher frequencies¹⁰. Thus, at the higher frequencies the radio emission is relatively weak and only the most intense, that is Io-controlled, events are detectable by the conventional low-gain radiometers. The weak, predominantly non-Io-controlled emission at these same high frequencies is generally undetectable, except by large antenna systems. On the other hand, at low frequencies, that is those nearer to the 8-MHz spectral peak, both the strong Io-controlled and weak non-Io emission can exceed the radiometer detection thresholds, leading to the specious conclusion that Io control has diminished. The apparent correlation between frequency and Io control is thus seen to result from a selection effect owing to the admixture of increasing amounts of non-Io emission as the observing frequency (and hence relative antenna detection threshold) is

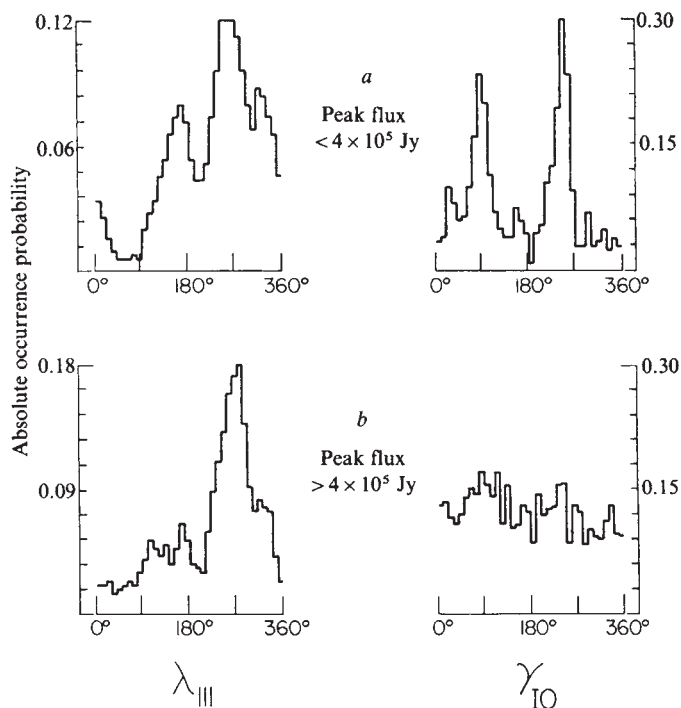


Fig. 2 A total of 491 h of 18-MHz jovian radio emission, organized as in Fig. 1, with high and low intensity components represented by a and b, respectively. Each component contains approximately equal amounts of activity. The intensity threshold in this case is 4×10^5 Jy. Observations were made at the University of Florida Radio Astronomy Observatory between 7 May 1964 and 8 May 1967, totalling 4,280 h of actual monitoring. As at 26 MHz, Io strongly modulates the high intensity emission, but the low intensity emission seems free of any Io control.

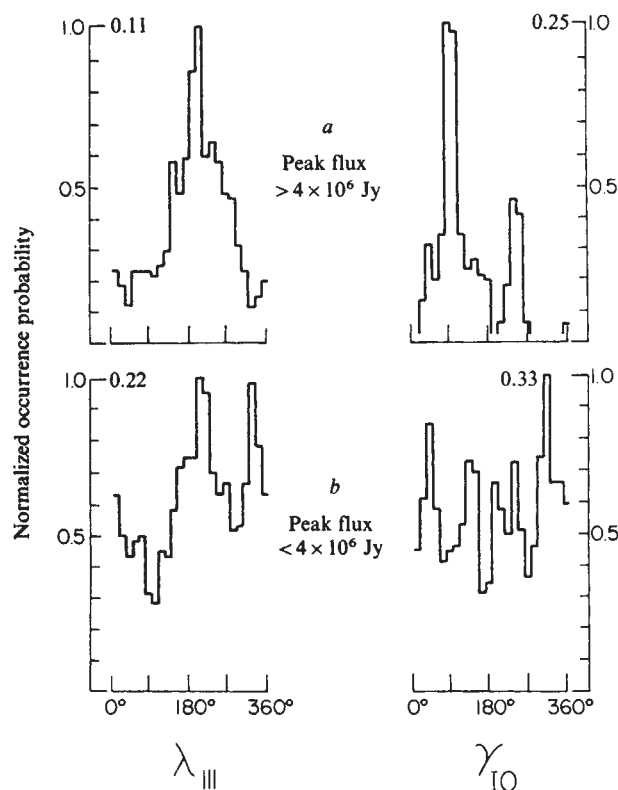


Fig. 3 A total of 138 h of 10-MHz jovian radio emission, organized as in Fig. 1. Here the low intensity component contains approximately three times as much activity as the high intensity component. The total observing time was 758 h. The observations at 10 MHz were made at the Maipo Radioastronomical Observatory in Chile in exceptionally good observing conditions between 18 May 1963 and 20 January 1964. The flux density threshold was set at 4×10^6 Jy. Qualitative agreement with the results at both 18 and 26 MHz is clear. Io modulation is evident in the case of the high intensity emission but nonexistent in the case of the relatively weak emission.

reduced. Primarily because the aforementioned low-frequency RAE 1 satellite observations were made with a relatively insensitive radiometer, it is not surprising that strong Io control was observed.

These results bear directly on the question of the radial extent of the Io-controlled source region. Those theories¹¹⁻¹⁴ which specifically address the problem of the stimulation mechanism itself generally postulate emission at or near the local electron gyrofrequency. Based on this assumption, an Io-controlled source location can be inferred from the Pioneer-modelled ambient magnetic field¹⁵ and, most importantly, from the observed frequency range of the Io-controlled emission. The present study infers a contiguous Io-controlled source region which, rather than being limited to the near-surface planet, extends out several jovian radii along the Io flux tube. Detailed satellite studies are now being made to define more precisely the spatial extent of the source.

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Si-rich Fe-Ni grains in highly unequilibrated chondrites

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Some Fe-Ni grains in the Bishunpur L3 chondrite have Si contents in the range 1.3–4.5 mg per g (mole fractions of 0.003–0.009); the only previous report of Si in ordinary chondrite metal is a lower limit of 0.7 mg per g for metal in the Leighton H4 chondrite¹. The Bishunpur Si contents are 1.1–4 times that found in a large metal grain in the Murchison CM2 chondrite². As whole-rock systems both meteorites have high degrees of oxidation and consist of unequilibrated, essentially unmetamorphosed mineral assemblages. Thus the Si was not introduced into the metal during metamorphic reheating, but rather during nebular condensation². Grossman *et al.*² calculated that the Si mole fraction of 0.0024 in the Murchison grain required a total nebular pressure $\geq 10^{-5}$ atm, which is higher than that generally inferred for the CM formation location. We show here that improvements in the Si activity coefficients and plausible constraints on the nucleation of mafic silicate condensates allow Si mole fractions of 0.003 at nebular pressures of $< 10^{-6}$ atm. Thus such chondrites could have formed over a wide range of distances from the Sun.

Bishunpur is one of the three most unequilibrated chondrites³⁻⁵ (Krymka and Semarkona are the others), and thus best preserves the record of processes occurring in the portion of the primitive solar nebula where the ordinary chondrites formed. Our data were obtained by electron microprobe; experimental details and Bishunpur's opaque phases are reported elsewhere⁶. All observations were made on polished thin sections 2359-1, 2359-2, and 2359-3 loaned by the Smithsonian Institution. Contrary to previous suggestions², we did not find that fluorescence of Si in adjacent silicates was a problem: (1) Si was below our detection limit of 0.4 mg per g in all metal grains except those listed in Table 1 and in two others having variable Si contents apparently reflecting the presence of Si-rich inclusions; (2) the problem is least where it is expected to be greatest, as regions of the Si-bearing metal that abut silicates have Si contents below our detection limit.

Figure 1 shows photomicrographs of three of our eight Si-bearing metal grains. Figure 1a, using transmitted light and partially crossed polars, shows an FeS-metal aggregate which is embedded in an olivine grain in the matrix (no. 39 in Table 1). The olivine grain, possibly a fragment of a chondrule, contains numerous opaque inclusions and it also displays undulatory extinction indicating distortion by shock. Not resolvable in this photomicrograph is a Ca-Al-rich glass having low Na and Fe concentrations. Such glass enriched in refractories is relatively common adjacent to Si-bearing metal, indicating that refractory materials are preserved within these chondrules. The large opaque inclusion is shown by reflected light in Fig. 1b. Its outer region is FeS and other minor phases and the interior is metal with a Ni content in the kamacite range. The sulphide gives the impression of having formed by corrosion of a metal grain.

Another FeS-metal assemblage (no. 19 and Table 1) is embedded in an olivine pyroxene chondrule that also contains

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two other Si-bearing metal assemblages and numerous small, uncorroded, spherical metal grains (Fig. 1c). We cannot detect Si in the uncorroded grains and the Si content of the corroded grain drops below the detection limit at those places where there is no FeS shield (upper right corner, near the dark embayment on lower right). Both the Si-bearing metal and the surrounding FeS contain numerous inclusions too fine to analyse. Some metal grains were not free enough of inclusions to be suitable for analysis. The areas whose compositions are listed in Table 1 were uniform in Si though an occasional point showed a high Cr content, perhaps reflecting the presence of FeCrO_4 or FeCrS_4 . The Si we determined is probably in solid solution in the Fe–Ni, but we cannot completely rule out the presence of uniformly distributed submicroscopic grains of Si-rich phases such as perryite, $(\text{Fe}, \text{Ni})_2(\text{Si}, \text{P})_5$. As such a phase would have formed by low-temperature exsolution, its presence would not complicate the following discussion of how the Si was originally introduced into the metal.

Figure 1d shows the only Si-bearing metal that was not enclosed by olivine (no. 78 in Table 1). It is located in a thin layer of opaque, amorphous matrix with large porphyritic chondrules on either side. The entire assemblage is $480 \times 320 \mu\text{m}$, thus its area is about quadruple that of the largest of the grains embedded in olivine. The metal grains are more-or-less randomly distributed within the sulphide. They show little variation in Ni, Co, and Si, but Si drops below the detection limit at grain edges. As in the other assemblages, there is a Ca, P and Cr-rich layer between the metal and the sulphide. Unlike the grains within chondrules, this assemblage does not have a rounded outline, nor does it have the inner metal, outer sulphide configuration. Despite the differences, the compositional and petrological evidence indicates that this grain assemblage is genetically related to those found inside chondrules. It may have been freed of silicates before the chondrule melting process, or it may have been a fragment broken out of a chondrule. The preservation of reduced Si despite its siting in the matrix can probably be attributed to its large size.

Table 1 shows the compositions of the six Si-bearing Fe–Ni grains for which precise data could be obtained, and the Fe/(Fe+Mg) ratios in the host olivine. All grains are Cr-rich, but there is no correlation between Cr and Si or between these

and other compositional parameters including olivine composition. One relevant observation is Cr zoning; Cr increased in concentration towards the grain edge. This implies that Cr was being reduced and diffusing into the grain before diffusion ceased. A possible interpretation is that Si was the agent reducing the Cr. All Ni concentrations are in a relatively restricted range of 34–40 mg per g, a composition that falls within the α stability field at temperatures between 550 and 1,100 K.

Summations in the FeS around the metal grains are often <980 mg per g reflecting the presence of the numerous fine inclusions mentioned above; even in areas of essentially pure FeS ($\text{Fe} + \text{S} > 980$ mg per g), Ca, Cr, P and Si totalling 8–33 mg per g are observed.

Clearly, the FeS shell has either introduced the Si or preserved Si that entered the Fe–Ni at higher temperatures. As FeS cannot reduce silicates to Si, and because the only observed evidence for an oxidation–reduction reaction involves Si oxidation, we conclude that the latter is correct. We can think of two mechanisms by which the FeS shell could preserve the reduced state of Si during the mild metamorphism experienced by Bishunpur. It could either act as a barrier to diffusion of oxidant or Si, or it could act as a reducing buffer which consumed any oxidant (Fe^{2+}) released by the surrounding silicates. The reducing agent might have been $(\text{Fe}, \text{Ni})_3\text{P}$ or Cr^{2+} . Diffusion at the low metamorphic temperatures experienced by Bishunpur probably limited any oxidant generation to very small amounts.

In unequilibrated chondrites the metal inside chondrules is significantly less equilibrated than the metal in the matrix⁴. Thus it is not surprising that seven of the eight Si-bearing grains that we observed are inside chondrules or inside olivine fragments that may have originated as chondrules. As the chondrules were either completely molten or high-degree partial melts, their temperatures at formation were surely $>1,250$ K, the FeS–metal eutectic temperature, it follows that the Si-bearing metal–FeS assemblages were also molten. It therefore seems probable that the concentric geometry (FeS as an exterior shell) and corroded appearance were produced during solidification and a very brief period of annealing. As the Si was not oxidized by FeO from the silicates during this process, it follows that the chondrules cooled very rapidly following formation.

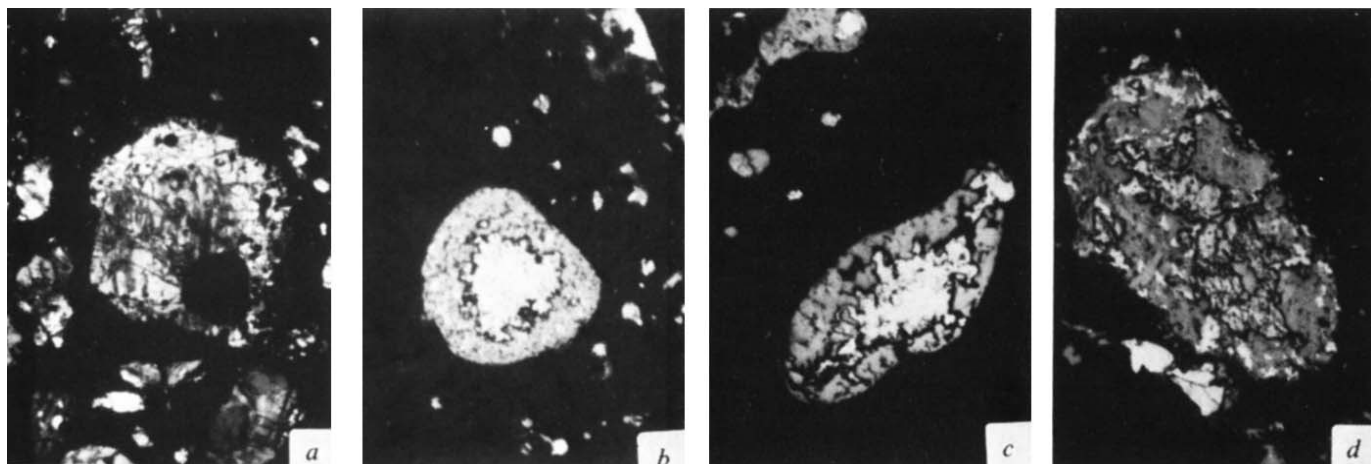


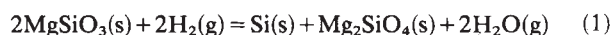
Fig. 1 Cr, Si-rich Fe–Ni grains in Bishunpur. *a*, Transmitted light photo showing a $760 \times 950\text{-}\mu\text{m}$ olivine crystal. The olivine displays undulatory extinction, probably indicating shock, and it contains numerous glassy inclusions of variable composition, but all strongly enriched in Ca and Al. The small opaque inclusions are mainly metal; the large one ($190 \mu\text{m}$) is shown by reflected light in *b*. The white area is metal (no. 39 in Table 1) and the outer rim consists of FeS. The irregular outline of the metal suggests that the sulphide formed by corrosion of the metal. *c*, Reflected light view of a $97 \times 310\text{-}\mu\text{m}$ metal–sulphide assemblage (no. 19 in Table 1) inside a chondrule. In the interior, the Si content is constant (3.7 mg per g) but drops below the detection limit near edges which are not coated in FeS. A fine-grained FeS-rich material, located between the metal and sulphide, is rich in Ca and P. The outer sulphide is relatively inclusion-free and contains Ca, Cr and minor P and Si. *d*, A large $320 \times 480 \mu\text{m}$ metal–sulphide assemblage (no. 78 in Table 1) surrounded by a thin layer of matrix and sandwiched between two large porphyritic chondrules. In contrast to the other grains, the metal (white, irregular) is randomly distributed in the sulphide. The Si content drops below the detection limit at the edge of each metal grain.

Table 1 Compositions of Si-bearing metal and adjacent olivine in the Bishunpur L3 chondrite

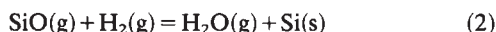
Grain	Points	Fe (mg per g)	Co (mg per g)	Ni (mg per g)	Si (mg per g)	Cr (mg per g)	Olivine (mol % Fa)	Petrographical setting
19	5	954	4.6	38	3.7	8.6	3.6	Separate grains in chondrule
20	3	933	4.5	40	1.3	10.0		
27	3	941	4.2	36	2.9	6.7		
39	5	948	8.0	40	2.0	2.0	1.4	Olivine fragment
62	6	950	6.4	36	1.6	5.2	—	Chondrule
78	6	946	6.5	36	3.4	6.2	—	Matrix

The 250×360-μm metal grain from the Murchison CM chondrite² contained 1.2 mg per g Si; there is no indication that it was surrounded by a sulphide shell. Considered as whole-rock chemical systems both Bishunpur and Murchison are highly oxidized. In Bishunpur ~50% of the Fe is present in the +2 state as oxides; in Murchison about 75% is in the +2 and +3 state as oxides. At equilibrium Fe-rich silicates or magnetite will readily oxidize Si. The large amount of oxidized Fe and the highly unequilibrated nature of both chondrites are strong evidence that the Si-bearing metal did not form by reduction during metamorphism.

The more plausible mechanism for producing Si-bearing metal is to produce it during the condensation of the cooling solar nebula. In an unfractionated nebula the Si activity in Fe–Ni will be controlled by reactions of the sort



if condensed silicates are the source of the Si. If gaseous SiO is the source of the Si the appropriate reaction is



The chief hindrance to the accurate calculation of Si mole fractions during nebular condensation is the inadequacy of available activity coefficient (γ_{Si}) data⁷. Sears⁸ used the function determined by Sakao and Elliott⁹ at temperatures of 1,373–1,623 K:

$$\log(\gamma_{\text{Si}})\alpha = 1.19 - \frac{7,070}{T} - \left(6.30 - \frac{18,300}{T}\right)X_{\text{Si}} \quad (3)$$

A study by Sakao *et al.*¹⁰ yielded $\log \gamma$ values about 0.15 more negative. Grossman *et al.*² used the Sakao–Elliott function and one covering the temperature range 873–1,273 K obtained by Roberts¹¹, we have not used the latter because it does not cover the range of condensation temperatures for Fe–Ni. The Sakao–Elliott data were obtained for Ni-free α (body centred cubic) phase. The Fe–Ni that condenses from the nebula has the γ structure (face centred cubic) and a Ni content >50 mg per g. We cannot predict the effect of Ni, but there is evidence that $(\gamma_{\text{Si}})_{\gamma}$ is lower than $(\gamma_{\text{Si}})_{\alpha}$. Wai and Wasson¹² reported that Si concentrations in the γ phase of the Tucson iron are 1.3–1.5 times higher than those in the coexisting α phase. However, equilibrium was not achieved in Tucson. (There is no definite γ phase in other highly reduced meteorites that are more equilibrated than Tucson; Keil¹³ found phases having 91–98 mg per g Ni in the Fe–Ni in EH Adhi-Kot and EL Hvittis, but the low Ni content of these grains leaves doubt that they are really γ .) The observed $\text{Si}_{\gamma}/\text{Si}_{\alpha}$ distribution ratio is probably a lower limit of that prevailing at the equilibration temperature of ~1,000 K; a plausible guess is that $(\gamma_{\text{Si}})_{\gamma}$ is about three times lower than $(\gamma_{\text{Si}})_{\alpha}$ at 1,000 K. Because γ should approach unity with increasing temperature, we will assume that the $\text{Si}_{\gamma}/\text{Si}_{\alpha}$ distribution ratio drops to 2 at 1,500 K. $\log(\gamma_{\text{Si}})_{\gamma} - \log(\gamma_{\text{Si}})_{\alpha}$ usually follows an $A + B/T$ relationship over moderate temperature ranges, so that equation (3) can be written:

$$\log(\gamma_{\text{Si}})_{\gamma} = 1.24 - \frac{7,600}{T} - \left(6.30 - \frac{18,300}{T}\right)X_{\text{Si}} \quad (4)$$

the absolute change of $\log \gamma_{\text{Si}}$ is assumed to be the same for γ and α metal.

Figure 1 shows the nebular condensation of Si for two models: (1) the thick curves show the metal compositions if Fe–Ni and mafic silicates condensed heterogeneously as soon as temperatures fell low enough to make the condensates stable; and (2) the thin curves show the compositions if metal condensed heterogeneously, but the mafic silicates condensed homogeneously with amounts of required undercooling¹⁴. The thermodynamic data set is the same as that used by Sears⁸, with the exception of the Si activity coefficient.

We suggest that the second model is the more appropriate one for the low-pressure regions of the nebula in which ordinary and carbonaceous chondrites formed. Evidence¹⁵ indicates that CM chondrites formed at solar distances ≥ 2 AU where nebular pressures were probably $\leq 10^{-5}$ atm, and ordinary chondrites at ~1–3 AU at pressures $\leq 10^{-4}$ atm. Although Blander and Abdel-Gawad¹⁴ and Blander¹⁶ argued that at low nebular pressures ($\leq 10^{-4}$ atm) nucleation inhibitions should result in Fe–Ni nucleating at temperatures well below those at which enstatite and forsterite condense, the opposite must have occurred for the Si-rich grains. If supersaturation of metal led to condensation of Fe as silicates or FeS, subsequently condensed metal should have had a Ni/(Fe + Ni) atom ratio well above the solar ratio of 0.054; but the Murchison ratio (0.064) is only slightly higher, the ratios in Bishunpur (0.030–0.038) distinctly lower. Moreover, there is no way to condense metal having high Si contents after Si has largely ($\geq 99\%$) condensed as silicates. If the nebula had a 'solar' $p\text{H}_2\text{O}/p\text{H}_2$ ratio of $\sim 5 \times 10^{-4}$, the Si concentration in subsequently condensed metal will be orders of magnitude lower than that observed in Bishunpur and Murchison. Thus, although we cannot rule out the condensation of other metal grains after silicate condensation, the evidence indicates that these Si-bearing grains condensed earlier than the bulk of the silicates. It has been proposed that refractory metal grains such as those embedded in refractory inclusions in CV chondrites could have served as condensation nuclei for Fe–Ni (ref. 17).

The carbonaceous and ordinary chondrite groups contain large quantities of Fe-rich silicates that probably either formed by direct condensation or by reaction of gaseous Fe with pre-condensed MgSiO_3 . Direct condensation avoids the need for mass-transport of Mg out of and Fe into these silicates, and may be more likely. Direct condensation implies a high degree of supercooling both for metal and silicates, consistent with our conclusion that most of the silicate condensation occurred at temperatures well below the equilibrium condensation temperature for Fe–Ni, and not inconsistent with the condensation of a minor amount of the metal at high nebular temperatures.

Figure 2 shows mole fractions of Si in Fe–Ni at nebular pressures of 10^{-4} , 10^{-5} , and 10^{-6} atm based on these alternative models. The positions are marked where $\text{Mg}_2\text{SiO}_4(\text{Fo})$, Fe–Ni(M), and $\text{MgSiO}_3(\text{En})$ become stable if equilibrium holds, and the point where silicate liquid (L) becomes stable in the constrained equilibrium model of Blander and Abdel-Gawad¹⁴. The heavy curves depict the Si/(Fe + Ni + Si) trajectories in equilibrium conditions. Maximum mole fractions reach 0.006, 0.002 and 0.001 at 10^{-4} , 10^{-5} and 10^{-6} atm, respectively. The results at 10^{-4} and 10^{-5} atm seem to agree with those of Grossman *et al.*². The light curves show the Si mole fractions for metal grains condensing heterogeneously (in equilibrium conditions) while the condensation of the major silicates has not occurred

because of nucleation constraints; the extensions of these curves below the estimated silicate nucleation temperatures (L) are shown dashed. If our second model is correct, then the $Si/(Fe+Ni+Si)$ ratios of 0.012, 0.007 and 0.003 are reached before silicates condense at nebular pressures of 10^{-4} , 10^{-5} and 10^{-6} atm, respectively. If this is the correct model for the formation of Si-bearing grains, the Murchison grain could have formed at nebular pressures of 10^{-6} atm or slightly lower.

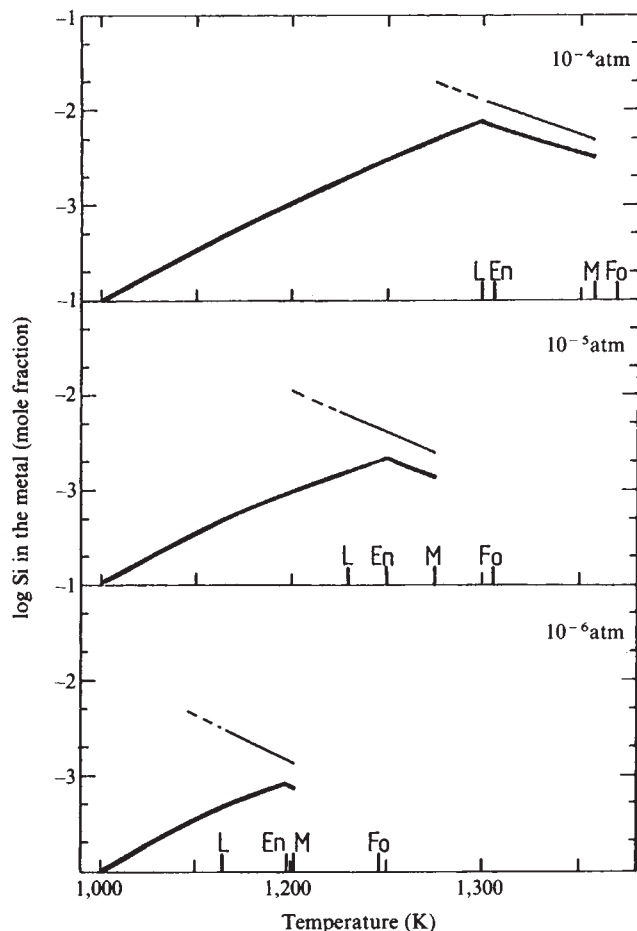


Fig. 2 Mole fraction Si in Fe-Ni condensing in a cooling nebula of solar composition at total (p_{H_2}) pressures of 10^{-4} , 10^{-5} and 10^{-6} atm. The heavy curve shows the Fe-Ni composition if both Fe-Ni(s) and Mg_2SiO_4 (s) condensed according to simple thermodynamic equilibrium. The light curve shows the Si content of the metal if the metal condenses according to the equilibrium calculations but silicates condense only after 70–85 °C of supercooling. Also indicated are the nucleation temperatures of Mg_2SiO_4 (Fo), Fe-Ni(M), $MgSiO_3$ (En) and a silicate liquid (L).

Is it reasonable that high Si contents could be preserved after mafic silicates start to condense? In some cases the Si was oxidized by $H_2O(g)$ and entered silicates that nucleated on the surfaces of the metal grains. On the other hand, uncondensed $SiO(g)$ is a more readily available source of Si, and rapid formation of silicates around a metal grain could remove the metal from contact with the gas and preserve its Si content, just as the refractory-rich metal grains are preserved in the inclusions of CV chondrites^{17,18}.

Studies of highly unequilibrated chondrites have taught us that if a phase can form in certain solar-nebula conditions it will probably eventually be found in these remarkable meteorites.

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Dead Sea Scroll parchments: unfolding of the collagen molecules and racemization of aspartic acid

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The parchments of the Dead Sea Scrolls were prepared from the skins of animals, mainly sheep and goats^{1,2}. They were copied and hidden between the beginning of the first century BC and AD 70³. Since then and until their discovery in the late 1940s by local Bedouins, they were located in caves close to the north-western shore of the Dead Sea⁴. Having been handled by various intermediaries for periods of up to 8 yr, most of the intact scrolls are housed in the Israel Museum, Jerusalem. We have examined small pieces ($\leq 25 \text{ mm}^2$) from these scrolls, together with random fragments without any writing, to study some of the chemical changes which have occurred in the past 2,000 yr or so. Using X-ray diffraction and racemization analyses, we found that a major degradative change is due to the unfolding of the collagen molecules to form gelatin and that the degradation generally occurred many centuries ago.

Inspection of these parchments clearly differentiates lighter and darker areas (Fig. 1). The latter are generally located close to the edges and are obviously less well preserved. Under the scanning electron microscope, the damaged edges are generally seen to be more massive and gel-like than the better preserved areas, in which fibres can usually be identified. The IR spectra of Dead Sea Scroll fragments show a marked decrease of the $3,330\text{-cm}^{-1}$ amide A band absorption as compared with modern parchments, consistent with the interpretation of a loss of collagen structure⁵.

We have developed a technique using X-ray diffraction to determine semiquantitatively the relative proportions of collagen and gelatin in parchment (Fig. 2). Only very small samples (a few mm^2) are required. The method is non-destructive and contaminating inorganic minerals generally do not interfere. In parallel, we have determined the ratios of the left-handed (L) and right-handed (D) isomers of aspartic acid, present in scroll fragment hydrolysates. Living tissues are generally composed of L-amino acids only. After death, conversion to the D-configuration slowly occurs to form eventually equilibrium mixtures of D- and L-isomers. Conditions influencing this process include the ambient temperature, the time elapsed, the pH, the protein amino acid sequence and the molecular conformations^{6–8}. Thus, measurements of the D/L ratio may provide additional insight into the rates and conditions in which the chemical changes have occurred. Aspartic acid

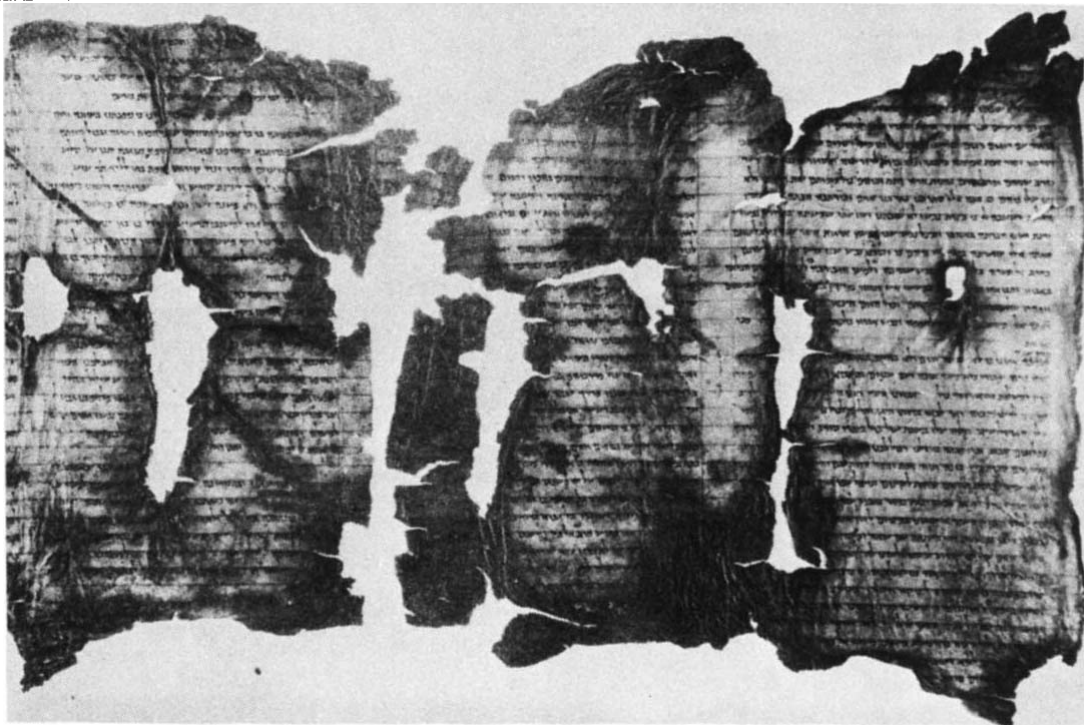


Fig. 1. The Thanksgiving Scroll soon after unrolling.

undergoes racemization relatively rapidly⁹ and is, therefore, well suited for this study.

The collagen to gelatin indices (C:G) and the ratios of D- and L-aspartic acid (D/L Asp) of the samples, are listed in Table 1 and plotted in Fig. 3. The C:G indices of Dead Sea Scroll parchments fall between the values for fresh rat tail tendon collagen and gelatin. On hydrolysis, both collagen and gelatin yield a small amount of D-aspartic acid, with gelatin showing more of the D form. Most of the scroll parchments, however, contain more D-Asp than is generated during the analytical procedure. The general distribution of the data in Fig. 3 shows that samples enriched in collagen contain only small amounts of D-Asp, whereas those enriched in gelatin have a range of D-Asp concentrations from insignificant to ~30%. In some cases, dark, poorly preserved edges were compared with lighter, better

Fig. 2 Optical densitometer traces of X-ray diffractograms of collagen, gelatin and Dead Sea Scroll parchments. *a*, Gelatin; *b*, random fragment 32; *c*, random fragment 35; *d*, collagen. The parchment surfaces were mechanically cleaned and then X-rayed in a Debye-Scherrer camera for 8–24 h depending on the size of specimen. Optical densitometer traces through the centre of the film were made using a microdensitometer (Joyce, Loebel, model MKIII B) in standard conditions (slit width, 0.8 mm; sensitivity, 8; chart speed, 7; 0–1.0 absorbance wedge). The C:G indices were estimated by dividing the sum of the slopes for the 10-Å peak by the sum of the slopes for the 5-Å peak (the 10-Å peak is both higher and narrower in collagen than in gelatin, whereas the 5-Å peaks are quite similar for the two materials and provide an internal standard for each photograph, so that the C:G index does not depend on experimental conditions such as specimen size and exposure time). The standard deviation on repeat preparations of two samples (32 and 35; Table 1) is 0.4, but is probably much larger in samples composed predominantly of collagen, due to the difficulty of estimating the slope of the 10-Å peak. The results are not very dependent on exposure time, unless the film is either very light or very dark. Relatively fast scanning on the densitometer and a wide slit effectively results in a trace with minimal interference from the sharp mineral lines. The C:G index values are on a relative scale. Interpolation between collagen and gelatin curves indicates that 25, 50 and 75% collagen compositions correspond to ~1.4, 2.8 and 5.0, respectively, on the C:G scale.

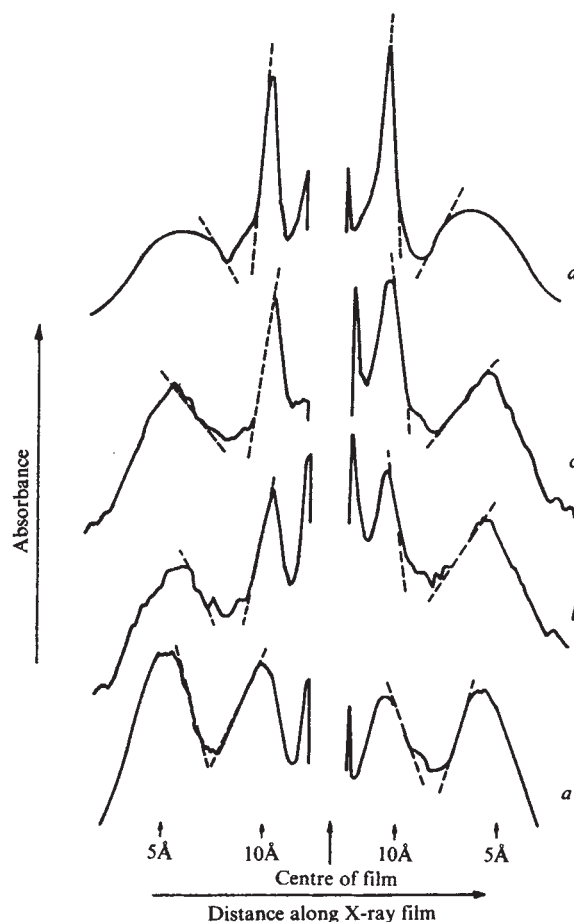


Table 1 C:G index and D/L Asp of the samples analysed

Sample no.	Sample description	C:G	D/L Asp	Sample location and notes
1	Collagen	10.2	0.05, 0.04	Washed and dried rat tail tendon collagen
2	Gelatin	0.7	0.08, 0.09	Rat tail tendon denaturated at 100 °C for an hour in water
3	Modern parchments	9.9, 9.8, 12.5‡	trace	Different pieces prepared according to current Jewish rituals
4	17th century Dutch book cover	7.6	0.05	
5	Isaiah (St Marks) (representative)*	0.6	0.07	
6	Isaiah (St Marks)	1.0, 1.5‡	0.08	
7	Isaiah (St Marks) (repair patch)	3.5	0.14	Located on patch directly beneath sample 6
8	Isaiah (St Marks) (repair patch edge)	2.3	0.14	
9	Psalms (representative)	3.7	0.13	
10	Psalms	1.6	0.21	Located at dark edge a few cm from sample 9
11	War Scroll	1.6	0.12	Samples 11 and 12 located on first panel which is uniformly darker than other panels
12	War Scroll	1.1	0.18	
13	War Scroll	2.3	0.12	Located on second panel
14	War Scroll	0.6	0.13	Located on third panel
15	War Scroll	1.9	0.14	Located on last panel
16	Temple (representative)	1.9	0.25	Fragment that fell off during unrolling; scroll is backed with rice paper
17	Temple	1.3	0.29, 0.27, 0.31‡	Pieces taken from gelled top of scroll
18	Twelve Minor Prophets (ref. 17§, Pl. LXII) (representative)	3.1	0.08	
19	Twelve Minor Prophets (ref. 17, Pl. LXII)	2.0	0.31, 0.30†	Located 0.5 cm from sample 18
20	Twelve Minor Prophets (ref. 17, Pl. LXXI)	3.2	0.04	Best preserved area
21	Twelve Minor Prophets (ref. 17, Pl. LXXI)	2.1	0.15	Located a few cm from sample 21
22	Thanksgiving Hymns	1.4	0.22	5 samples span a 1.7-cm long section from a lighter area to a dark area
23	Thanksgiving Hymns	1.0	0.20	
24	Thanksgiving Hymns	1.2	0.20	
25	Thanksgiving Hymns	0.8	0.21	
26	Thanksgiving Hymns	1.6	0.22	
27	Fragment 891 (representative)	3.9	0.09; 0.11†	
28	Fragment 891	1.8	0.09	Located a few cm away from sample 30
29	Fragment 891	1.0	0.13	Dark edge adjacent to sample 31
30	Book of Jonah (fragment)	4.5	0.17	
31	Book of Jonah (fragment)	5.7	0.10	
32	Random fragment 4Q922C	3.8, 4.3, 3.6, 4.4‡	0.14, 0.11‡	
33	Random fragment 11Q988A	3.9, 3.4†	0.24	
34	Random fragment 11Q988B	2.0	0.17	
35	Random fragment 4Q920	5.5, 6.0, 6.4, 5.8, 6.6‡	0.08	
36	Random fragment	2.7	0.14	

* By visual criteria at least, samples designated 'representative' resemble the major portions of the total scroll.

† Two separate analyses of the same preparation.

‡ Separate preparations and analyses.

§ Ref. 17.

preserved areas from the same scroll. It was generally observed that the better preserved samples are enriched in collagen and contain less D-Asp (see, for example, samples 7 and 8; 9 and 10; 16 and 17; 20 and 21; 27, 28 and 29). In some cases, samples located within 1 cm of each other have D/L Asp ratios which span almost the entire range encountered (Table 1; Fig. 3).

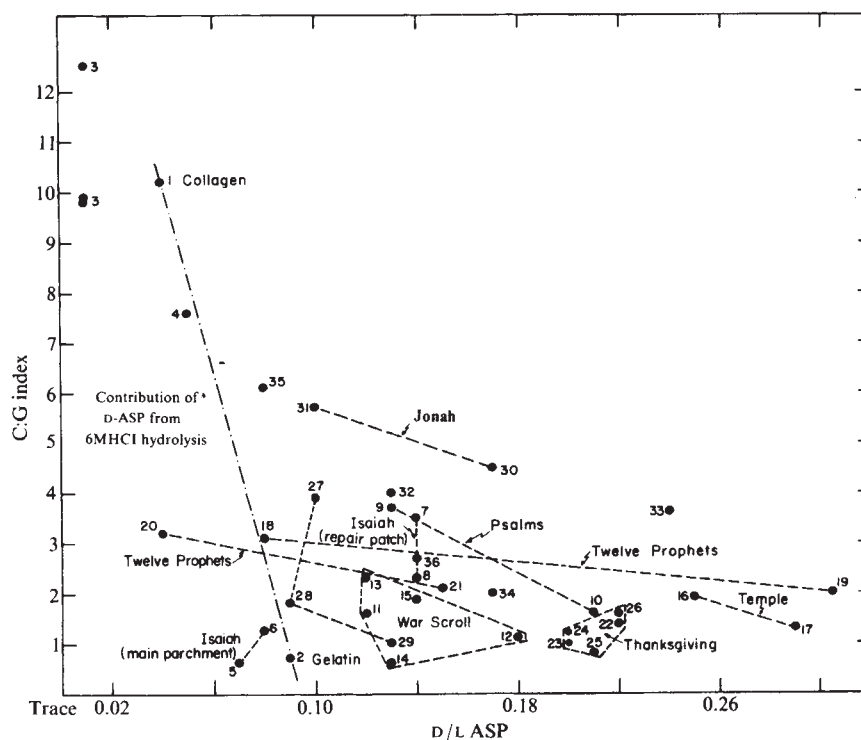
Using different skins in one scroll and/or various parchment preparative treatments could conceivably influence the C:G index and the D/L Asp ratios. The first panel of the War Scroll, although uniformly darker than the other panels, is not significantly different from the rest of the scroll with respect to the two parameters measured (Table 1). The Isaiah Scroll had been repaired by the addition of a patch of parchment in one corner. In this case, the patch is enriched in collagen and contains more D-Asp than the main scroll parchment, which curiously shows no significant concentrations of D-Asp. Note also that the Temple Scroll, which has a light appearance and is relatively pliable, has surprisingly high concentrations of gelatin and D-Asp.

The unfolding of collagen to gelatin seems to be one of the major chemical changes which these parchments have undergone. The dark borders have suffered more than the light interior sections (Fig. 1). Furthermore, during unrolling it was observed that the inner sheets were better preserved than the outer sheets¹⁰, suggesting that an external environmental agent, probably water, influences the conversion of collagen to gelatin.

Collagen is more stable in drier conditions¹¹ and the low relative humidity in the area of the caves was probably a major factor responsible for the preservation of these materials. The micro-environment around the inner portions of the scrolls themselves was probably particularly dry as the outer layers probably trapped incoming water vapour. Extreme dryness also reduces the racemization rate of amino acids¹². Fungal and bacterial activity may also have been absent for this reason. The sharp boundaries observed between light and dark areas are generally paralleled by marked variations in C:G proportions and D/L Asp ratios. This suggests that major water-accumulation events occurred sporadically during limited periods. Continual exposure to humidity would result in gradual variations in these two parameters due to a slowly expanding area in which collagen is unfolding.

It is easier to envisage racemization reactions and the consequent incorporation of D-amino acids occurring in gelatin, which has a largely disordered structure, than in the regular conformation of collagen. Indeed, the dominance of negative slopes for the connecting lines of Fig. 3 shows that, for different pieces of the same scroll, significantly higher proportions of D-Asp correspond to higher proportions of gelatin. Thus, the time of formation of gelatin from collagen should influence the ultimate D-Asp content measured. Some of the samples have accumulated considerable amounts of D-Asp (see Table 1 and Fig. 3). As fossil organic materials generally require hundreds

Fig. 3 The index plotted against D/L Asp of the samples analysed. For racemization analyses, ~2–5 mg of parchment were used. The parchment surfaces were mechanically cleaned, soaked in double distilled water for 2–3 h at room temperature and then transferred to 0.5 M acetic acid for 4 h at room temperature to remove the more soluble contaminants. The samples were hydrolysed in redistilled 6M HCl *in vacuo* at 150 °C for 40 min. Amino acid compositions of the random fragments closely resemble the amino acid composition of collagen. After extensive drying, the hydrolysates were derivatized to *N*-trifluoroacetyl isopropyl ester as described previously¹⁵. The samples were dissolved in ether and passed through a silica column (Merck, 200 mesh) in a Pasteur pipette. The aspartic acid enantiomers were separated by gas-liquid chromatography using a stainless capillary column coated with *N*-lauroyl-L-valine *t*-butylamide¹⁶. To confirm the identity of the D-Asp peak some of the samples were also chromatographed on the corresponding D-valine phase, which showed the expected peak reversal. Where large amounts of D-Asp were found, trace amounts of D-alanine and occasionally D-leucine, were also observed. The relative proportions of D- and L-Asp were measured by peak height. The reproducibility of the analyses is 5–10%. However, a significant portion of this variability may be due to sample heterogeneity. The ratios have not been corrected for the amounts of D-Asp generated during hydrolysis.



or thousands of years to accumulate significant amounts of D-Asp⁶⁻⁸, it is reasonable to assume that most of the parchment fragments must have suffered collagen to gelatin denaturation many centuries ago, rather than in the comparatively short period since their removal from the caves. A careful visual comparison of photographs of scrolls taken soon after being unrolled 30 yr ago (Fig. 1) with the same scrolls in January 1980, revealed no noticeable differences in the distribution of light and dark areas. As at least the scroll fragments excavated by archeologists were found to be buried beneath sediments⁴, the early damage may be due to the lack of a protective covering for some time after the scrolls were deposited in the caves or even, in part, be due to handling by the original readers. A corollary of our arguments might be that samples with relatively high gelatin but low D-Asp contents, such as the Isaiah scroll (see Fig. 3), were damaged more recently. However, this presupposes a more or less constant rate of racemization unaffected by differences in preparative treatments and microenvironmental conditions for different portions of the scrolls. The lower gelatin but higher D-Asp contents of the repair patch (see Fig. 3), which has presumably been attached to the Isaiah scroll for the past 2,000 yr, suggests that such factors probably do affect the rate of racemization.

Our study shows that the transformation of collagen to gelatin is one process which causes significant deterioration of the parchment. Optimal preservation conditions, which minimize the rate of transformation, should be established. As a guideline, the minimal melting temperatures of Dead Sea Scroll collagen¹³, about 22 °C, and a relative humidity of 65% (above which uptake of moisture by collagen increases very rapidly)¹⁴ should not be exceeded. The C/G index can be used in this regard to monitor any possible further deterioration, as well as to perform long-term simulation experiments.

Compared with various fossil materials, on which age determinations by racemization were made, parchment is a relatively well defined proteinaceous material. Nevertheless, our data show a wide range of D/L Asp ratios for different scrolls emanating from the same period and often even for different parts of the same scroll. Before attempting age determinations of parchment by the D/L Asp ratio, we need to understand better the mechanism of the changes involved and to develop an appropriate sampling procedure.

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High winter concentrations of SO₂ in the Norwegian Arctic and transport from Eurasia

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Since July 1977, the Norwegian Institute for Air Research has been studying trace gases and aerosols in the atmosphere at Bear Island, an Arctic site located at 74° N and 19° E. Although Bear Island lies well north of the Arctic Circle, the warm Norwegian Sea gives it an annual mean temperature of -1.8°C , considerably warmer than at many other Arctic locations (Barrow, Alaska, for example, is 350 km farther south but has an annual mean of -12.2°C). In summer, Bear Island is surrounded by open water; in winter there is open sea to the south and west and pack ice to the north and east. Atmospheric samples are taken 20 m above mean sea level and 2 m above local ground: high-volume filters are taken three times a week and analysed for various elements by atomic absorption, neutron activation and wet chemistry; sulphate and sulphur dioxide are measured daily by a method similar to that of Johnson and Atkins¹, using low-volume (16 m^3) prefilters for sulphate and KOH-impregnated afterfilters for SO₂. The collection efficiency of this method for SO₂ has been tested extensively². Results for the high-volume samples have been reported³; here we discuss the SO₂ data, which seem to indicate that during winter there is efficient transport from Eurasian midlatitudes, due at least in part to long atmospheric residence times in and around the Arctic.

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During summer, Bear Island has a narrow temperature range (typically 0 – 10°C at noon) and persistently low concentrations of SO₂ (at or below the detection limit of $0.2\text{ }\mu\text{g m}^{-3}$). For example, from May to October 1978 there were only 10 days with SO₂ concentrations $>0.4\text{ }\mu\text{g m}^{-3}$. During winter, however, the weather becomes more variable (temperatures range from 0 to -30°C); SO₂ concentrations (and those of aerosol) often become much higher. For example, from November 1977 to April 1978 there were 65 days with SO₂ concentrations $>0.4\text{ }\mu\text{g m}^{-3}$. Concentrations of SO₂ are also highly episodic in winter, with about three to five irregularly spaced pulses per month. Pulse duration can vary from one day to more than a week. Between pulses, SO₂ concentrations are at the summer values, whereas daily mean concentrations in pulses reach 1 – $6\text{ }\mu\text{g m}^{-3}$. Figure 1 records SO₂ concentrations from mid-December 1977 to mid-April 1978. *c* indicates probable contamination by the local diesel generator, deduced from wind observations every three hours.

Three of these pulses were associated with warm air from the south (0 to -5°C), but 10 were associated with colder air (-10 to -30°C). Wind direction during the cold-air episodes was typically northeastern at the surface and N-NE at 1,500-m elevation (which eliminated the possibility of local contamination from the north-west causing the high SO₂ values); wind speed was often high ($>10\text{ m s}^{-1}$) during cold-air episodes. During the period of Fig. 1, nearly every pulse of cold air at Bear Island had high SO₂ concentrations.

There is considerable evidence that this 'Arctic' SO₂ is derived from distant pollution sources, probably the Eurasian midlatitudes. (1) There is no correlation between the presence or elevation of local temperature inversions and SO₂ (or aerosol) concentrations, as would be expected if local sources dominated. With E-NE winds, there may be either ground-based temperature inversions or near-adiabatic profiles to a few hundred metres with an inversion above that. With W-SW winds there are usually surface-based inversions. (2) Aerosol species such as sulphate, vanadium and lead usually peak at the same time as SO₂, and have high enough concentrations (2 – $4\text{ }\mu\text{g m}^{-3}$, 1 – 3 ng m^{-3} and 5 – 10 ng m^{-3} , respectively) to suggest that they have come directly from polluted areas. Of these, at least

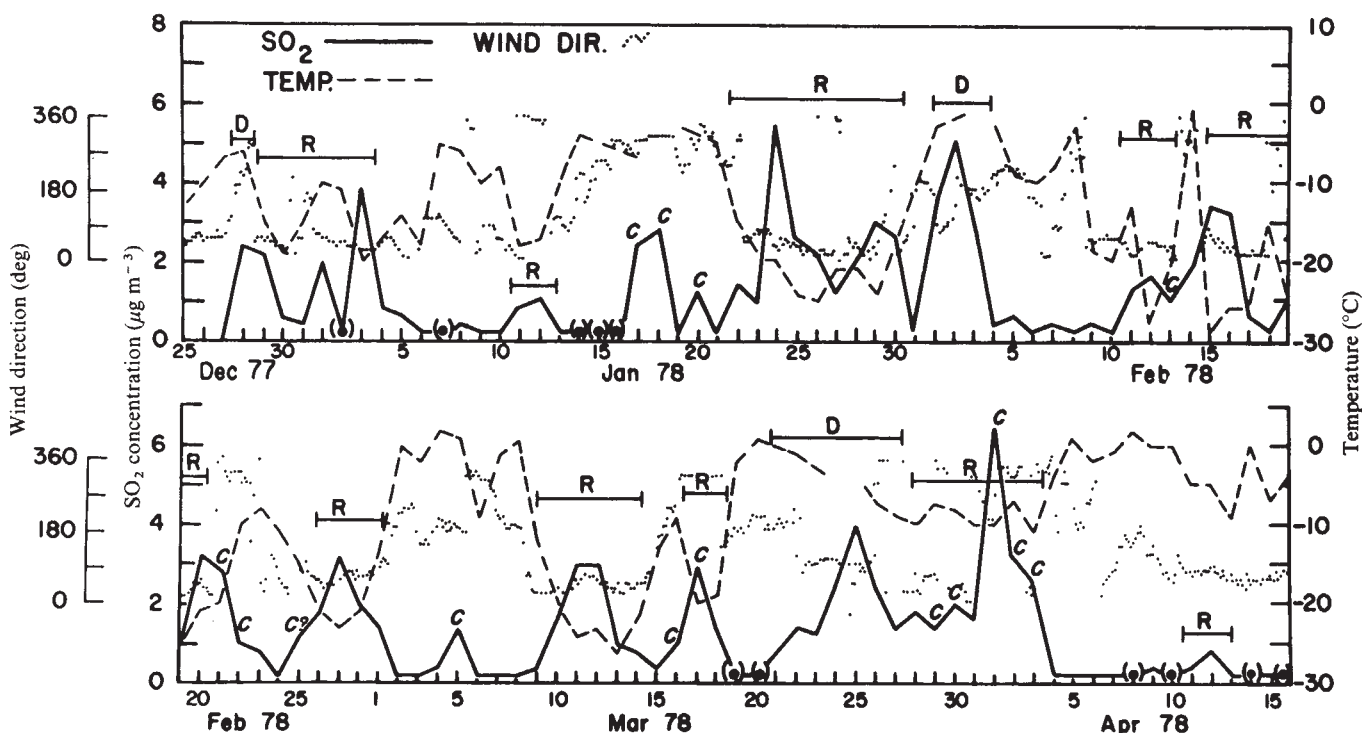
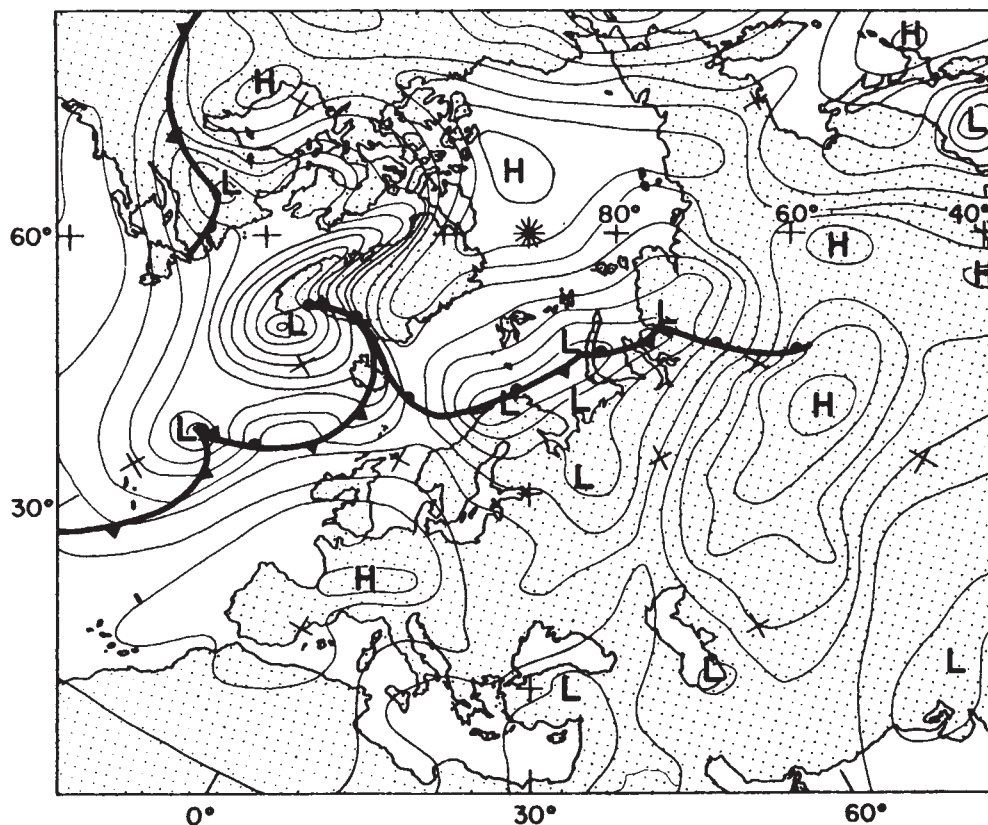


Fig. 1 Daily SO₂, temperature and wind direction at Bear Island, December 1977 to April 1978. D, Direct flow; R, return flow; c, probable contamination by local diesel generator.

Fig. 2 Simplified surface synoptic situation for 10 March 1978, typical for cold-air episodes of SO₂ at Bear Island. H, High pressure; L, low pressure.



vanadium has its main pollution sources in midlatitudes⁴. Occasionally, however, peaks of aerosol are not accompanied by peaks of SO₂. (3) Preliminary trajectory analysis indicates that during cold-air episodes, air flows to Bear Island around the eastward and northward sides of one or more low-pressure centres located over or just north of Scandinavia and the western USSR. A typical surface synoptic situation, for 10 March 1978, is shown in simplified form in Fig. 2. Surface-geostrophical five-day back-trajectories (which represent air flow between the surface and 1,500 m) for five high-SO₂, cold-air days at Bear Island during winter-spring 1978 are shown in Fig. 3. These trajectories, which are only rough guides to how the air masses could have travelled, indicate that for four of the five days, the air reaching Bear Island did seem to curve cyclonically around the low-pressure system, coming first from the region between Novaya Zemlya and the Taymyr Peninsula, and before that from farther south in the western USSR. Allowing for large-scale lateral diffusion, many of the densely populated areas of the western USSR and Europe are included within travel times of roughly 5–10 days. We call this the 'return-flow' pathway from Eurasia to the Norwegian Arctic.

A similar pathway to the Arctic was proposed earlier^{4,5} for aerosol. The return-flow pathway is different from the 'direct-flow' pathway, where air flows northward from Europe to Bear Island, on the eastward side of a low-pressure centre located more to the south or west, and creates a warm-air episode³. In Fig. 1, these two types of air flow, as determined from surface meteorological maps, are indicated by 'R' and 'D', respectively.

The reason for the different character of the trajectory of 24 January 1978, which apparently came from the north, is not clear. The general synoptic situation at that time was similar to those for the other four cases of high SO₂; consequently, we think that the high SO₂ of 24 January came ultimately from the south as well. This illustrates the uncertainties of meteorological analysis in the Arctic, where weather stations are spaced widely.

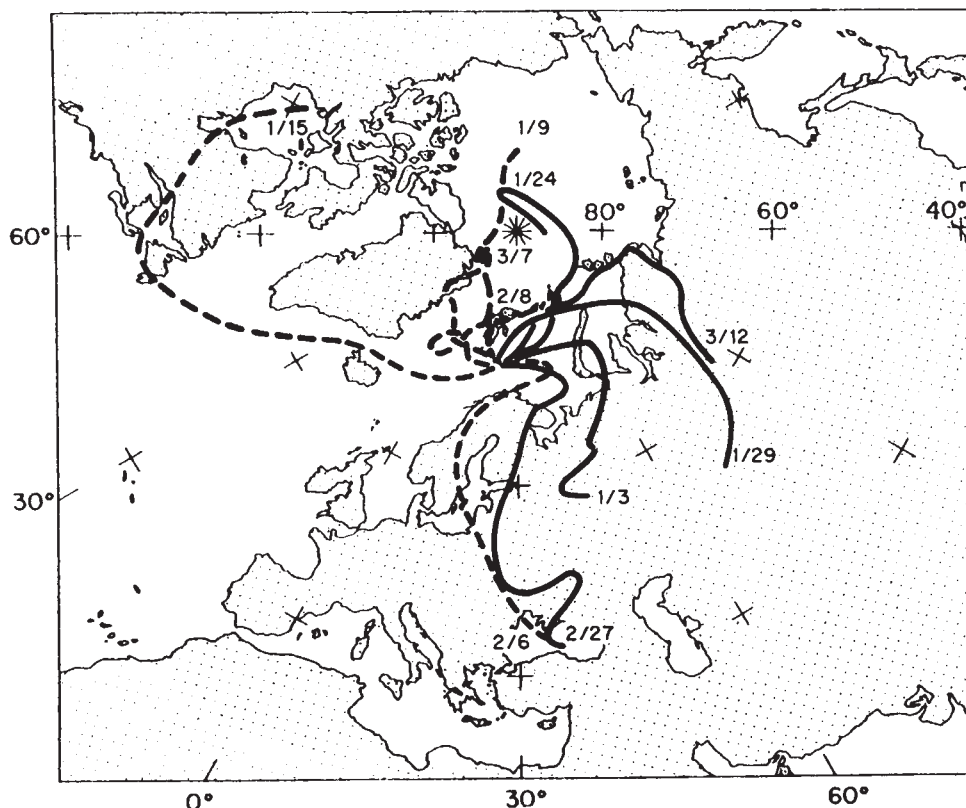
The five trajectories for days of low SO₂ came from other directions. Three originated north and east of Bear Island, one came from North America via the North Atlantic, and one even

came from Scandinavia and Europe. The trajectory of 6 February, which seemed to bring very low SO₂ from Europe, is another problem. It came a few days after a strong direct episode, but with considerably weakened pressure systems by the time it had reached northern Norway. Aerosol concentrations were still one-third to one-half of their values at the peak of the episode, but for some reason, SO₂ had decreased to more than an order of magnitude below its peak.

It seems, however, that rapid transport along the return-flow pathway from Eurasia to the Arctic is insufficient to explain the high winter SO₂ there; long atmospheric lifetimes also seem to be required. Transport times from Eurasia to Bear Island of 5–10 days, initial SO₂ concentrations of 30 $\mu\text{g m}^{-3}$ (mean winter values for the industrialized countries of Europe^{6,7}, probably an overestimate for Europe as a whole), dilution of the polluted air mass by a factor of four during transport (derived from simulations of aerosol transport to Bear Island, probably an underestimate), and a final SO₂ concentration of 3 $\mu\text{g m}^{-3}$ at Bear Island require that mean residence times during transport be 5–10 days. If the residence time increases during transport, as is likely, an initial value of 2–5 days at the source is still needed to explain the measured SO₂ concentrations. In contrast, currently accepted atmospheric residence times for SO₂ in Europe are 1–2 days^{8–11}. Thus, winter residence times for SO₂ in Eurasia would seem to be up to five times longer than annual mean values, and in the Arctic of the order of weeks.

There are good physical and chemical reasons for these long residence times. In Eurasia, the residence time for SO₂ is determined primarily by the rate of dry deposition to the surface, secondarily by precipitation scavenging, and to a still lesser extent by the rate of oxidation to sulphate. In Arctic (or cold continental) winter conditions, all these rates should be very slow, because: (1) The rate of dry deposition decreases with increasing stability of near-surface air and surface smoothness; low values have been measured over snow¹². (2) Wet deposition of SO₂ should be small during winter in the Arctic because of the slight precipitation there, typically only 5 mm per month. (3) Heterogeneous oxidation of SO₂ in Arctic cloud droplets ought

Fig. 3 Five-day surface geostrophic back-trajectories for cold-air episodes (solid line) and periods of low concentration (broken line) during winter 1977–78. Dates of arrival at Bear Island are indicated.



to be very slow because of the low temperatures, the low frequency of clouds, their restricted vertical extent, their small liquid–water content, and the meagre amounts of trace elements such as Mn and V available as catalysts (30–40 times less than in midlatitude air masses). This oxidation rate may decrease as an air mass ages, because acidity produced in the droplets limits the subsequent solubility of SO_2 . (This decrease ought to be greatest over oceans and cold continents, where little NH_3 is available to neutralize the increasing acidity (C. Brosset, personal communication).) (4) Lastly, homogeneous oxidation of SO_2 by the OH radical ought to be extremely slow during the Arctic night because of the lack of direct solar radiation.

Similarly long lifetimes for aerosol in and around the Arctic have been deduced recently⁴. When combined with the long lifetimes for SO_2 , they begin to explain how gaseous and particulate sulphur can be efficiently transported northward from midlatitudes during winter and dispersed over the entire Arctic.

This case illustrates just how sensitive the atmospheric rates of transformation and removal of the various forms of sulphur may be to climatic properties. In 1977 the Dubrovnik workshop on atmospheric sulphur¹³ recommended that attention be given to these seasonal and regional variations for the sulphur system. Now that long-range transport of gases and aerosols through highly different climatic zones is known to be a regular feature of the atmosphere, such attention becomes mandatory, and should be extended to other species.

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The UK wave energy resource

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Previous estimates^{1,2} of wave energy around the United Kingdom have been made by extrapolating measurements from a few sites to the whole UK seaboard. Here directional wave spectra are used from a numerical wave model developed by the Meteorological Office³, to make estimates which are verified where possible by observation. It is concluded that around 30 GW of power is available for capture by wave energy converters: when estimates of converter spacing and efficiency are considered an average of about 7 GW of electrical power could be supplied. This resource estimate is smaller than previous ones, though consistent with them when factors such as the directional properties of waves and the likelihood that converters will be sited near coasts are included.

The first reliable wave measurements were made by weather-ships at Ocean Weather Ship Station 'India' in the North-west Atlantic, 710 km west of the Outer Hebrides. The power density at this location has been estimated at between 80 kW m^{-1} (ref. 4) and 91 kW m^{-1} (ref. 2), to give a UK resource of about 120 GW (ref. 1). In 1976, the Institute of Oceanographic

Sciences⁵ installed a waverider buoy 18 km west of South Uist, in 42 m of water. This has supplied the standard wave climate for evaluation of wave energy schemes^{6,7}, and the expected long-term average power density is 48 kW m^{-1} (ref. 6). In estimating the resource, account must be taken of the directional properties of waves: all the measurements referred to above have been of water-surface elevation at a point, and refer to the total power crossing a vertical cylindrical surface of 1-m diameter in the sea from all directions. Thus, the power density falling on a line of converters whose normal is at an angle θ to a given wave direction is $P \cos \theta$ where P is the power density measured by a non-directional instrument. Power usually arrives from a range of directions; this is referred to as a 'spread' sea. For a given orientation of a line of converters, the incident power per unit length is the integral of the product of the power flux in each directional component of the incident waves and their $\cos \theta$ factors. The ratio of this power to the non-directional power flux as defined earlier is termed the 'directionality factor', and is less than unity. The incident power density P crossing a line in the sea is then

$$P = p\delta \quad \text{kW m}^{-1}$$

where

$$p = \int_{-\pi}^{\pi} p(\theta) d\theta \quad \text{kW m}^{-1}$$

is the incident power density from all directions and

$$\delta = \frac{1}{p} \int_{-\pi/2}^{\pi/2} p(\theta) \cos \theta d\theta$$

is the directionality factor, usually integrated over waves crossing the line from one side only, as few wave energy converters absorb significant wave energy from behind. (This is not usually a significant restriction as near the coast few waves are generated travelling away from land.) If the converter line is oriented to intercept the maximum possible power (that is it faces the 'best direction'), then the directionality factor is a measure of the range of wave directions present. Off the Hebrides, for example, the best direction is close to west and the directionality factor is ~ 0.76 .

Data used here are from the Meteorological Office depth-dependent wave forecasting model, developed by Golding³. In this model, surface winds over the sea are used to calculate wave spectra. Wind data are from the Meteorological Office 10-level atmospheric model, and wave fields are calculated on a 50-km grid covering the North Sea and most of the North Atlantic. The effects of energy loss and spreading mechanisms such as wave

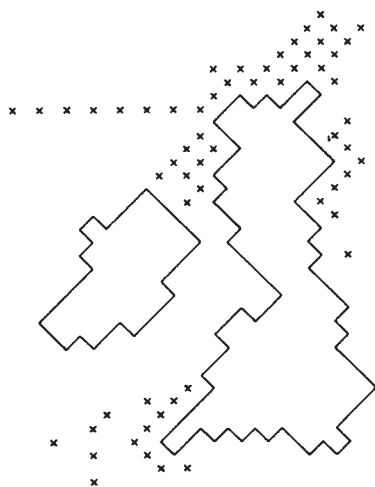


Fig. 1 The UK as it appears in the model. x, Gridpoints where the archived wave spectra have been analysed; data for a gridpoint near OWSS 'India' have also been analysed.

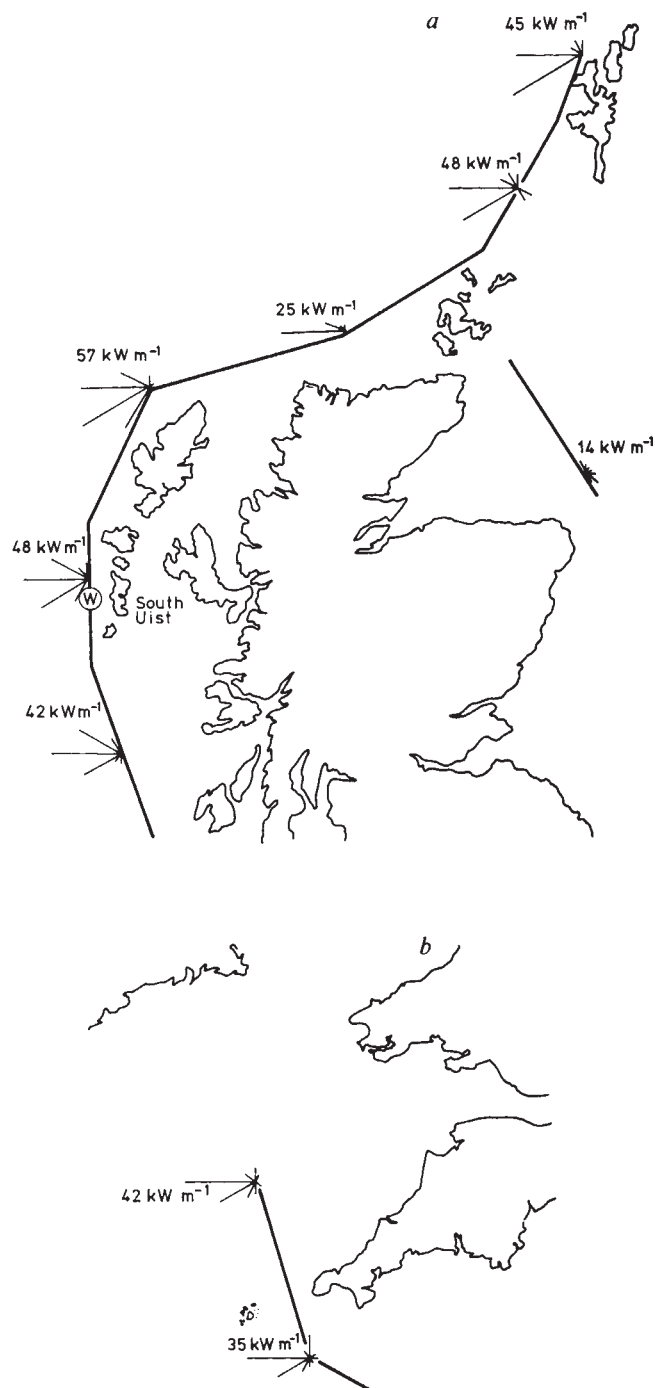


Fig. 2 a, Wave power at various gridpoints around the Scottish coasts. The numbers give average non-directional power density over the two years of data and the 'crows-feet' indicate the directional breakdown. The length of the direction bars in each crows-foot is proportional to the average power density arriving from a 30° sector centred on that direction. W, The location of the South Uist waverider. b, Wave power around the south-west of England. The heavy lines represent locations where it has been suggested that wave energy converters might be sited.

breaking, refraction and an idealized model of bottom friction are included. The resulting directional wave spectra are calculated every 30 min and archived every 12 h, and comprise spectral estimates at six frequencies (0.05–0.15 Hz) for each of 12 directions (that is, a 30° sampling interval) for every grid-point. The integrals referred to earlier have been calculated by summation over those directions. Results used here are from December 1977 to September 1979, the first two years of the model's operation. The gridpoints selected for analysis are

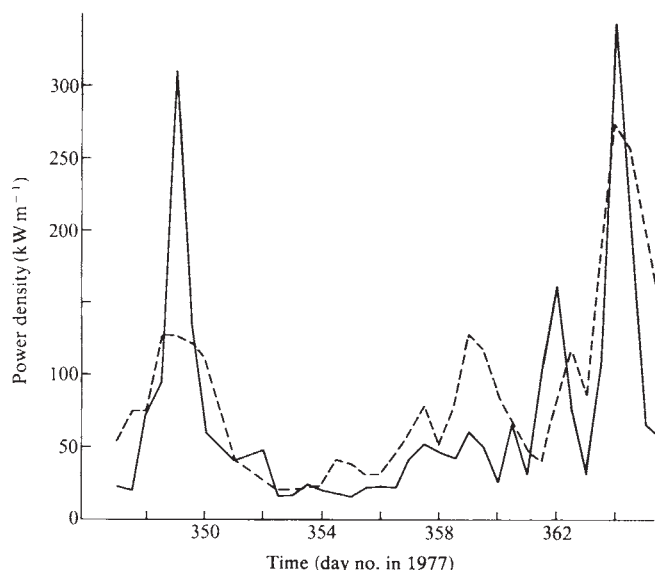


Fig. 3 Wave power density during part of 1977: measured by the waverider buoy at South Uist (solid line); results from the nearest model gridpoint (dotted line).

shown on Fig. 1. Evidently a 50-km gridpoint spacing is not fine enough to reproduce the convolutions of our coasts. To increase the accuracy of the model near the coast, the following procedure was adopted. Gridpoints were drawn on the 'real' maps (Fig. 2) at their correct positions where possible. If this was on land on the real maps (but in water in the model) or behind a real island which could not be reproduced in the model, then that gridpoint was displaced slightly when drawn on Fig. 2. In all cases, when calculating power levels the only directions used were those from which gridpoints as drawn on Fig. 2 could accept energy. This modification matches the coarse grid of Fig. 1 to the detailed coast shown in Fig. 2. It is applied after the wavefield has been computed, and does not affect refraction around headlands due to changes in bottom topography.

Figure 3 shows a time series of power for the South Uist waverider and the nearest model gridpoint, for 20 days in 1977. There are three points of interest. First, the model power levels are greater than the measured values in low-sea states, but less in high-sea states. Second, there is evidence of time shifts between the model and the waverider measurements, and finally high power levels develop sooner and die away more slowly in the model. These effects mean that when instantaneous model results are plotted against measured values they show a large scatter: this is reduced significantly if values for several consecutive time periods are averaged before being plotted. Figure 4 compares measured values and model results for South Uist. Each point represents the average power over a 2.5-day period: simultaneous observations and model results were available for 140 days in 1978. Figure 4 shows that the model gives slightly higher power levels than the waverider up to $\sim 100 \text{ kW m}^{-2}$. Above this power level, the trend is reversed. The average power levels over the 140 days of the comparison were in very good agreement. The waverider measured 49 kW m^{-2} and the Meteorological Office model gave 52 kW m^{-2} . Note that the expected long-term average power level at South Uist is 48 kW m^{-2} (ref. 6), implying that the wave climate over the comparison period was representative of the long-term average. There are few other sites where direct comparisons are possible, because of the limited number of instrumental wave measurements made during 1978–79. If it is assumed that the wave climate in 1978–9 was representative of the long-term average, then the wave power at OWSS 'India' may be compared. Mollison *et al.*² give a weighted average power of 91 kW m^{-2} for this location: the nearest model gridpoint gives 96 kW m^{-2} . The Meteorological Office model is, therefore, accurate enough to

determine the average power levels around the United Kingdom.

It should be emphasized that the power densities given below refer to power crossing the heavy lines in Fig. 2. For comparison, the power densities shown on various locations in Fig. 2 are omnidirectional power densities. The north-west line extends for 820 km from Islay, around the Hebrides to the Shetlands. The average power flux crossing the line is 24 GW, at a density of 29 kW m^{-2} . This value is lower than that suggested by non-directional measurements such as the 48 kW m^{-2} shown on Fig. 2a for South Uist: one reason is that a large fraction of the line (Cape Wrath to the Orkneys) does not face the best direction. In contrast, the section of the line around the Hebrides up to Lewis could provide 12 GW at a power density of 39 kW m^{-2} . This section faces approximately the best direction, and its directionality factor is 0.73, implying that most of the wave energy arrives from due west. The north-east line is often^{4,7} drawn across the mouth of the Moray Firth, and is about 110 km long with an average power flux of 0.65 GW. The power density is only 6 kW m^{-2} and the directionality factor 0.52. These low values arise mainly from the absence of swell, which accounts for some 65% of the wave energy on the west coast⁶. Weather patterns in the North Sea are less consistent than in the North Atlantic, and the spread of wave directions is much larger in the North Sea. If the line were extended southwards by 340 km from Peterhead to Scarborough, the average power flux across the extension would be 1.9 GW, at a power density of 5.5 kW m^{-2} . The south-west line is 190 km long and the average power flux across it is 4.9 GW, at a density of 25 kW m^{-2} . This is comparable to the level off the Hebrides and supports the idea that a large fraction of the energy is received in the form of swell generated by depressions travelling across the Atlantic.

Wave power levels at OWSS 'India' are much higher than those at South Uist, close inshore. Figure 5 shows the variation of power with shore distance off the Hebrides. Curve *a* indicates total (non-directional) power density; curve *b* indicates the product of power times directionality factor, which is roughly constant over the whole range of distances. This suggests that the reason for higher power levels at OWSS 'India' is that the power arrives from a larger range of directions there, a conclusion supported by curve *c* which shows the power density arriving from directions centred on $240^\circ \pm 45^\circ$. This is actually

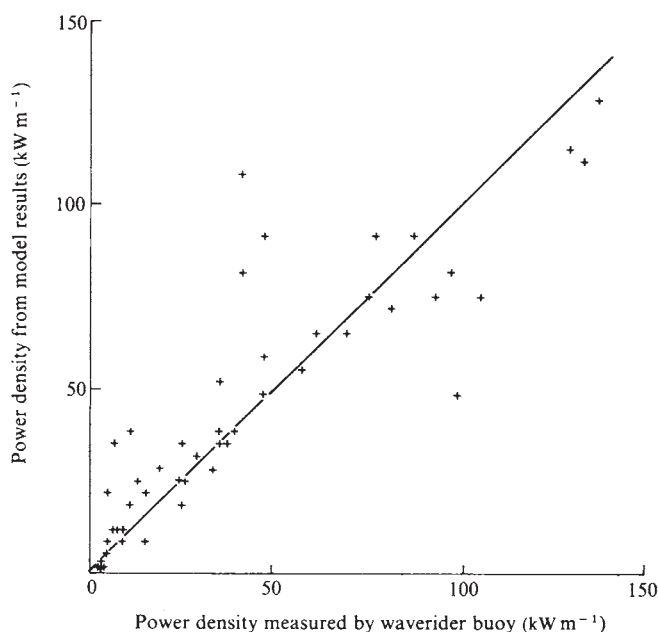


Fig. 4 Measured power densities plotted against model power densities. Each point represents the average values over a 2.5-day period, from a comparison of 140 days in 1978. The straight line has a slope of unity.

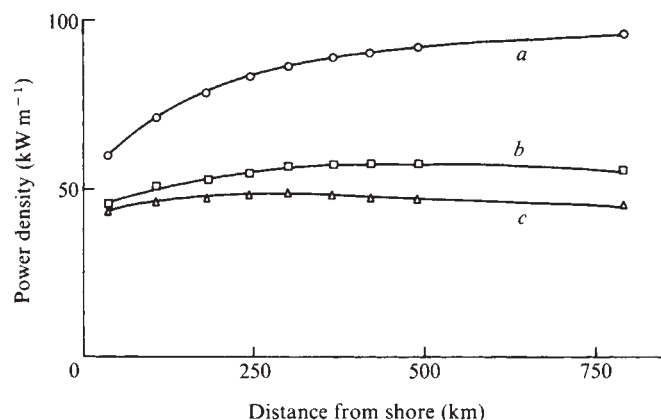


Fig. 5 Wave power as a function of shore distance, using the gridpoints extending westward from the Hebrides, shown in Fig. 1. Curves: *a*, the total power density from all directions; *b*, the product power times directionality factor; and *c*, the power arriving from directions centred on $240^\circ \pm 45^\circ$.

somewhat less at OWSS 'India' than at intermediate distances, probably because the westerly fetch for a typical depression is less there.

If ideal wave energy converters were sited on the lines shown in Fig. 2, the estimated wave energy resource would be 29 GW (annual average). Using this as an input figure, it is possible to estimate the contribution that such a scheme could make to the UK energy supply. There are two factors which would reduce the output of a large wave energy scheme. The first is that it would not be possible to install converters along the entire length of the lines shown in Fig. 2. Siting and mooring considerations imply that gaps must be left between individual converters; some parts of the lines could not be used as they intercept shipping lanes. It is difficult to quantify these factors at present. I assume here that 80% of the lines in Fig. 2 can be filled with converters.

The second factor arises from the low efficiency of converters. Most can capture 25% of the incident energy (averaged over a typical year), although it is possible that 30% can be achieved (S. Salter, personal communication). Taking values of 80% for the first factor and 30% for the second, the average power delivered would be 7 GW, ~25% of the CEEB's average power output. Siting converters further from the coast is unlikely to increase this substantially. As Fig. 5 shows, higher power further offshore comes from a larger range of directions; the power density crossing a line is largely independent of distance offshore. The suggestion that the resource could be significantly increased by deploying parallel lines of converters off the Hebrides is also debatable. Most of the wave power is in the form of swell, which would not be regenerated in a short fetch. The wind sea that might be regenerated could have a large directional spread, making capture less efficient because of the low directionality factor.

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North Pacific palaeoceanography: late Quaternary coiling variations of planktonic foraminifer *Neogloboquadrina pachyderma*

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Intraspecific coiling variations of the planktonic foraminifer *Neogloboquadrina pachyderma* (Ehrenberg) form an important micropalaeontological technique for the palaeoclimatic interpretation of late Cenozoic mid-latitude marine sediments. In all the modern oceans, this species preferentially coils dextrally in subtropical to temperate latitudes and sinistrally in subpolar to polar latitudes^{1,2}. Plankton³⁻⁵ and surface sediment studies⁶⁻²⁰ of the North Pacific clearly associate sinistral populations with subarctic water masses and dextral populations with temperate-subtropical regions. Here we show that down-core ratios of sinistral to dextral coiling, when correlated with oxygen isotopes, indicate major late Quaternary southward advances of the subarctic boundary (polar front) and the Oyashio Current during oxygen isotope stages 2, 4 and 6.

Advective circulation of western boundary currents such as the Gulf Stream and the Kuroshio Current serves to transfer warm saline water (and the associated warm moist air) to temperate latitudes of the Northern Hemisphere. The Kuroshio is probably the most important ocean current influencing the climate of eastern Asia and, ultimately, of western North America. The deep-sea sediments of the north-west Pacific record the history of the major trends of this climate pattern as the responses of the marine organisms to the ambient temperature.

The planktonic foraminiferal species *N. pachyderma* (Ehrenberg) is one of the most useful of such biostratigraphic tools for the interpretation of late Cenozoic marine sediments in high- and mid-latitudes. This species preferentially coils its test dextrally in subtropical to temperate latitudes and sinistrally in subpolar and polar seas^{1,2}. The general plankton distribution of *N. pachyderma* is known for the North Pacific³⁻⁵ and its occurrence in surface sediments has been mapped⁶⁻¹⁹. Here we describe the biogeographic distribution of the two coiling types for the northwestern Pacific from the total faunal analyses of more than 150 surface sediment samples²⁰ and the down-core coiling variations of 11 Quaternary piston cores (Table 1). The timing of the down-core coiling variations has been determined by correlation with the oxygen isotopic records of many of the cores and by the recognition of biostratigraphic datum levels in all of them.

Table 1 Cores used in the study

Core	N. latitude	E. longitude	Depth (m)	Isotopic refs	Palaeontological refs
V20-119	47°57'	168°47'	2736	23, 22	13, 20, 22
V21-146	37°41'	163°02'	3968	23, this work	14, 20
V28-294	28°26'	139°58'	2308	23	20
V28-304	28°24'	134°08'	2942	23, 25, this work	20, 25
V28-310	25°25'	125°38'	1579		20
V32-126	35°19'	177°55'	3870	This work	20
V32-128	36°28'	177°10'	3623		20
RC10-161	33°05'	158°00'	3587	23	17, 20
DSDP 310	36°52'	176°54'	3516		16, 18
DSDP 435	39°44'	143°48'	3401		24
DSDP 436	39°56'	145°34'	5240		24

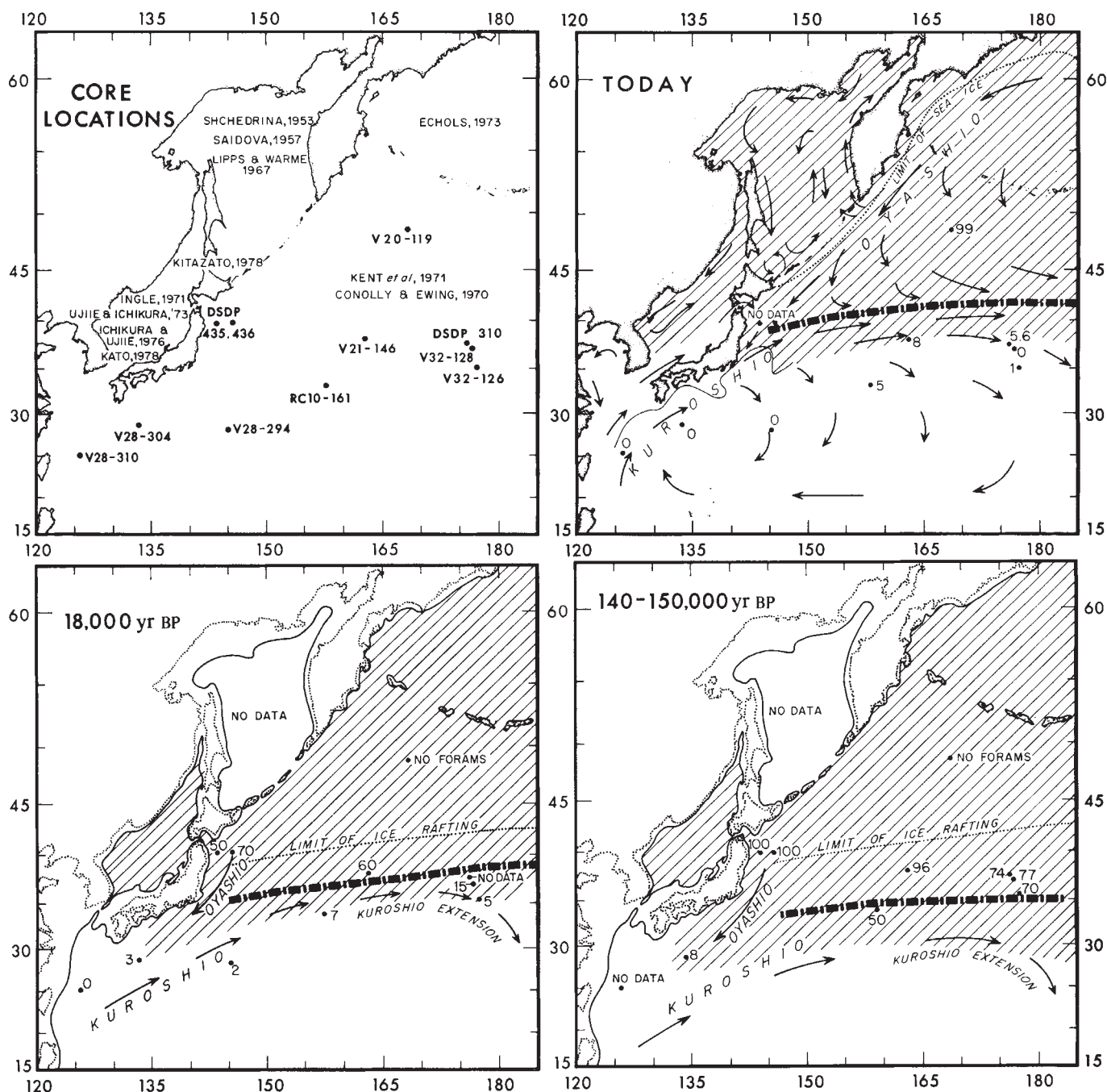


Fig. 1 Core locations and distribution of *N. pachyderma* in north-west Pacific. Upper left: core locations (see Table 1) and areas of previous study (refs 8–20, 33–36). Upper right: modern seabed percentage distribution of sinistral *N. pachyderma* relative to surface currents (arrows), position of subarctic boundary (heavy dashed line), and sea ice (dotted line). Lower left: 18,000-yr-BP position of subarctic boundary based on percentage of sinistral *N. pachyderma*, ice-rafted detritus²⁶ and sea-level drop of 120 m. Lower right: 140,000–150,000-yr-BP position of subarctic boundary.

The southern seabed limit of *N. pachyderma* is well defined along the western Pacific margin, where coring sites are closely spaced and the shallow depths of the locations minimize carbonate dissolution. The percentage frequencies of the species are very low at tropical sites, and frequencies do not exceed 1% south of 30° N. They account for 15–20% of the total fauna near Japan, however. Along the northern part of the Izu-Marianas Ridge and the area north of Taiwan, the dominant coiling mode is dextral. These individuals have 4.5–5 chambers in the final convolution forming a sub-rhomboidal outline; the surface of the test is non-spinose and coarsely reticulate. Dextrally coiled *Neogloboquadrina eggeri* (Rumbler) (= *Neogloboquadrina dutertrei* of some authors) is also present in these sediments, but can be easily recognized as it has more than five chambers in the

final whorl and an umbilical tooth in the apertural depression—*Neogloboquadrina pachyderma* has an apertural rim flush with the outer surface of the final chamber.

Near the confluence (about 35° N, 145° E) of the warm, saline and northward-flowing Kuroshio Current and the cold, low-salinity and southward-flowing Oyashio Current, small proportions (less than 2% of total fauna) of sinistral coiled *N. pachyderma* first occur in surface sediments together with the more abundant dextral forms. Further east beneath the axis of the Kuroshio Extension (35–40° N, east of 155° E), central North Pacific populations are predominantly dextrally coiled and account for about one-third of the planktonic foraminiferal assemblage of the surface sediments.

North of latitude 40° N and along the continental shelf of

Japan north of about 36° N, populations of *N. pachyderma* are almost exclusively sinistral, and planktonic foraminiferal assemblages are often nearly monospecific. Individuals typically have four chambers in the final whorl, giving the shell a quadrate outline; about 1–2% of the *N. pachyderma* tests are dextrally coiled but have the quadrate, four-chambered arrangement of the sinistral type.

There is some difficulty in associating an exact temperature with the changeover from dextrally coiled to sinistrally coiled populations of *N. pachyderma*. Because of the wide seasonal contrasts of sea-surface temperatures in the western North Pacific in mid-latitudes²¹, the changeover takes place where the annual range of temperature is 20 °C. There is no gradual shift of coiling direction, however, in the few sediment samples that have been recovered from this region: instead, there appears to be an abrupt switch from predominantly dextral coiling south of the subarctic boundary (polar front) to predominantly sinistral coiling north of it. Bradshaw³ mapped the maximum plankton abundance of *N. pachyderma* (probably quadrate sinistral forms) as lying between 44 and 48° N, corresponding to an annual sea-surface temperature range of 2–15 °C. In the north-eastern Pacific between 44 and 48° N, Banerji⁵ observed a change in plankton coiling directions at about 7–10 °C. These

data are in good agreement with the observations of Bé² that the species is typically found in Antarctic seas between –1 and 8 °C, and most abundantly below 2 °C. Sea-surface temperatures in the region of dextrally coiled *N. pachyderma* have an annual range of 15–25 °C, clearly indicating two separate biogeographic populations that may be mixed only along a narrow region of the subarctic boundary.

Although temperature is a classically cited influence on the distribution and diversity of marine organisms, additional factors such as salinity, dissolved oxygen and nutrient availability, are also important. The physical boundaries for planktonic populations appear to be those of water masses characterized as large regions of fairly homogeneous properties. Thus it seems unwise to define a boundary between the coiling modes of *N. pachyderma* based on sea-surface temperature alone; rather, it is more useful (until plankton studies can be made at different seasons) to consider the coiling types as characteristic of the adjoining subarctic (sinistral) and subtropical (dextral) water masses. On either side of the subarctic boundary, coiling populations are distinct, as are the oceanographic properties of the water masses. The area of mixed coiling directions then represents a narrow transitional region where the two populations are mixed, perhaps seasonally or in response to the

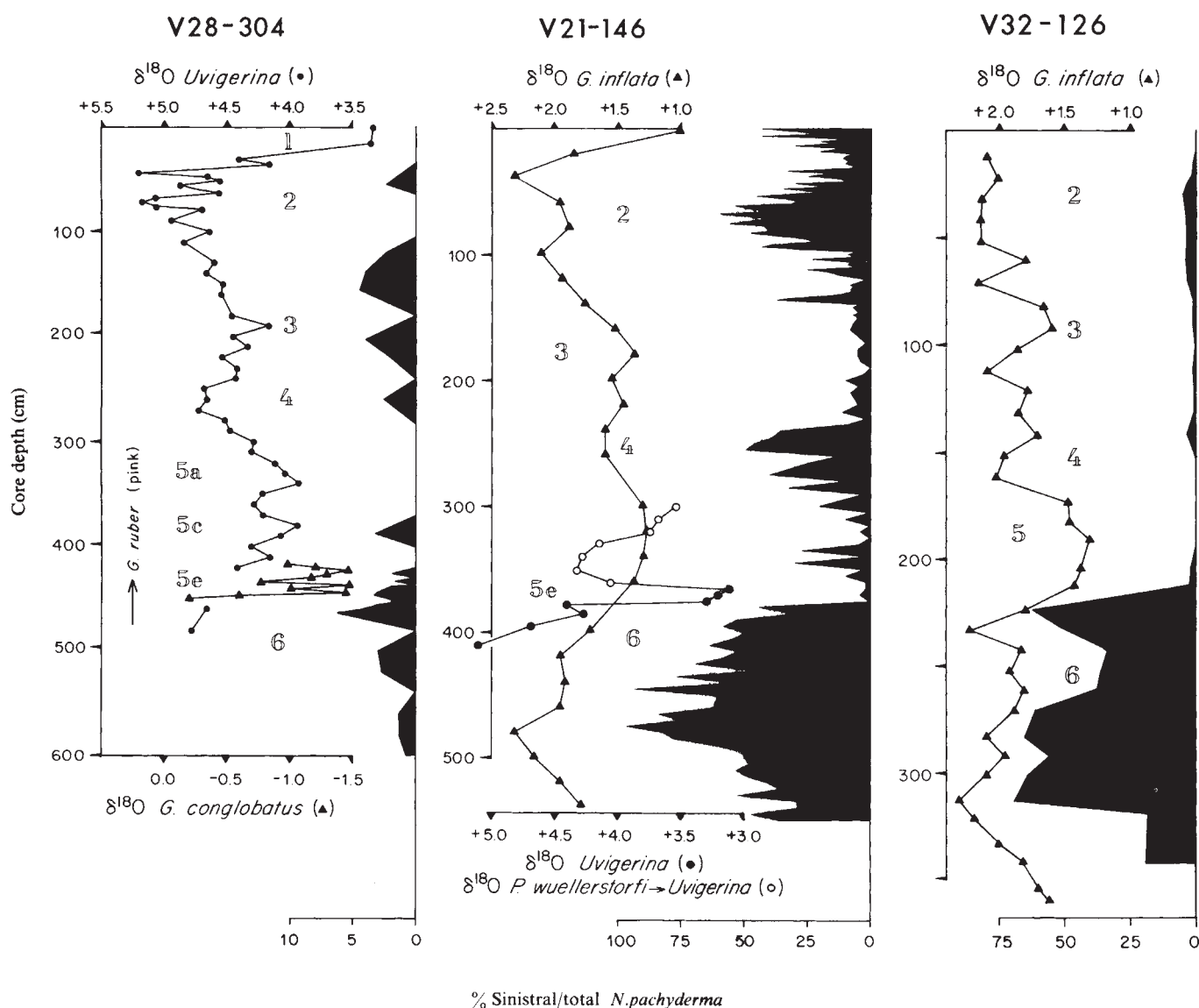


Fig. 2 Coiling ratio of sinistral to total *N. pachyderma* (shaded) versus oxygen isotopes (relative to PDB standard) for *Uvigerina* (solid circles), other benthic taxa (open circles) and planktonic taxa (solid triangles).

variable location of the many meanders and eddies along the confluence, and is further homogenized in the sediments by bioturbation over a long period of time.

Five of the six carbonate-rich piston cores (Table 1) were selected for both palaeontological and isotopic study because of their location on topographically positive features beneath the present-day axis of the Kuroshio Current and Extension; core V20-119 was retrieved from the Emperor Seamount Chain and represents a unique carbonate section available from the subarctic water mass¹³. Given the locations of the cores, we expected that down-core coiling variations of *N. pachyderma* could be interpreted as representing major fluctuations of flow within the Kuroshio gyre system, southward advances of the subarctic water mass or changes in carbonate preservation through time. (Total faunal analyses were made on plankton foraminiferal assemblages which will be described elsewhere.) Fluctuations of oxygen isotopic values are considered to monitor global ice volume^{22,23} and are believed to be virtually isochronous within the time resolution of the sedimentary record.

Piston core V21-146 (ref. 14), located just north of the present southern limit of the subarctic boundary, contains the most detailed record of coiling variations (Fig. 2). The strong sinistral peaks at 70 cm (60% sinistral) and at 450 cm (96%) are associated with oxygen isotope stages 2 and 6 respectively, while dextral maxima are well defined in isotopic stages 1, 3 and 5. The topmost centimetres of the core show a return to sinistral coiling which may be interpreted as marking the period of recent climatic cooling since the Climatic Optimum at about 6,000 yr BP. Although the planktonic foraminiferan *Globorotalia inflata* was used throughout the core for isotopic analyses, benthic foraminiferal taxa were used to provide the details across the 5/6 transition, including *Uvigerina* and *Planulina wuellerstorfi* (corrected to *Uvigerina* by +0.64%).

The piston cores located south of the present subarctic boundary [RC10-161 (Shatsky Rise)¹⁷, V32-126, V32-128 and DSDP Site 310 (Hess Rise)¹⁸] are marked by dextral coiling interrupted by sinistral maxima. In these cores, the dramatic shift of the coiling dominance of *N. pachyderma* at the glacial intervals so prominent in V21-146 is not apparent. Only the major sinistral peak in V21-146 during oxygen isotope stage 6 can be widely recognized in cores south of the present polar front. The stage 2 sinistral peak seen in V21-146 is weak in the southern cores; the stage 4 sinistral peak, which is only moderate in V21-146, often cannot be located.

Along the western margin of the Pacific coast of northern Japan, DSDP Site 435 (ref. 24) has a moderate ratio of dextral to sinistral coiled *N. pachyderma* near the top of the sequence, which gradually becomes entirely sinistral throughout the remainder of the downcore Pleistocene. Piston core V28-304, located beneath the axis of the Kuroshio south of Japan, shows very weak (<10% sinistral) peaks (Fig. 2); the stage 6 peak can be identified only by its isotopic position. Here, the details of the 5e/6 transition were determined by analysis of *Globigerinoides conglobatus* because of the curious absence of *Uvigerina* in that part of the core. A weak sinistral peak correlated with stage 2 is present, but there are also several additional sinistral pulses during stage 3, which was expected to be dominantly dextral. Core V28-310, located south-east of core V28-304, has no isotopic record; correlation with core V28-304 using the extinction of pink-pigmented *Globigerinoides ruber* at 120,000 yr BP (ref. 25), did not reveal any sinistral coiled *N. pachyderma* within the past 150,000 yr. Core V28-294, east of core V28-304, has a short stratigraphic record which does not extend to the pink *G. ruber* datum level and the oxygen isotope record identifies a weak sinistral peak in stage 2.

Core V20-119, from the centre of the subarctic water mass (Table 1), contains only sinistral coiled *N. pachyderma* which vary in absolute abundance (Fig. 2) from over 70,000 tests per gramme near the top of the core to totally barren intervals. These barren sections, when correlated to the oxygen isotope record²², are of glacial age. Although ice-rafted detritus is quite common in these same glacial sections²⁶, the sedimen-

tation rate does not differ greatly from that of the interglacial intervals. Moreover, carbonate dissolution does not account for the absence of both planktonic and benthic foraminifera for there is no increase in fragmentation before or after the barren region; in any case, dissolution studies²⁷ indicate that carbonate dissolution is more intense in interglacial periods than in glacial stages. In this case, however, diatom abundances are increased in the glacial sections²⁸, suggesting a shift in the areas of diatom and planktonic foraminiferal production.

In the CLIMAP 18,000-yr-BP reconstruction²⁹, we noted that the sea-surface cooling at the last glacial maximum was small over most of the Pacific in comparison with the Atlantic, although in the region near the Pacific coast of Japan sea-surface temperature gradients were quite steep. The down-core distribution of sinistral coiled *N. pachyderma* during isotopic stage 2 (as well as stages 4 and 6) supports the contention that the steepened gradient was the result of a southward advance of the polar front. In the modern ocean, the polar front overlies sediments containing 10–20% sinistral-to-total *N. pachyderma*; the water mass boundary (Fig. 1) for the past reconstructions has been drawn to correspond with such a ratio. Down-core increases in the sinistral-to-total ratio (Fig. 2) require that the subarctic boundary be moved to a location between 35–38° N during the stage 2 glacial and to about 32–35° N during the stage 6 glacial. South of Japan, core V28-304 marks the southernmost extent of the glacial advances of sinistral *N. pachyderma*, which implies drastic southward penetrations of the Oyashio Current (possibly as a submerged current³⁰) along the coast of Japan. Mollusc³¹ and pollen³² records of the Pleistocene of Japan indicate a cold dry climate as far south as Tokyo. A eustatic closure of the Sea of Japan eliminated the warm Tsushima Current and glacial sections from the Sea of Japan contain only sinistral coiled *N. pachyderma*^{33–36}.

The depositional centre of abundant sinistral *N. pachyderma* moved from near core V20-119 at about 50° N to about 45° N near core V21-146 during the late Pleistocene glacial advance (Fig. 2). The decrease in absolute abundances of both planktonic and benthic foraminifera in core V20-119 and the accompanying increase of diatoms in glacial isotopic stages 2 and 6 may indicate substantial circulation changes in the subpolar gyre resulting in southward shifts in the locations of pelagic habitats. Species common to modern Sea of Okhotsk sediments such as *Globigerinita uvula* and *Turborotalita quinqueloba*¹¹ become prominent members of glacial faunas of cores V21-146, V32-126 and V32-128. The dominance of sinistral coiled *N. pachyderma* at these sites is evidence for the prevalence of modern winter conditions over this area during most of the year.

In contrast, the interglacial maximum at isotopic stage 5e appears to have been somewhat warmer than today. As well as the dominance (up to 100%) of dextrally coiled *N. pachyderma* in both the tops and stage 5e of many cores (Fig. 2), there are many warm-water planktonic species in stage 5e samples, including *Globigerinoides conglobatus*, *Globorotalia truncatulinoides* and *Orbulina*. These species appear at low frequencies as far north as core V20-119, suggesting that the subarctic boundary may have been placed north of its present position, but the lack of foraminifera-bearing sediments precludes a firm conclusion.

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Occurrence of hydroxamate siderophore iron chelators in soils

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Although iron is abundant in soils (1-6%), it is often unavailable to plants because its solubility is dependent on pH and controlled by the low solubility of ferric oxides. Iron availability to plant roots may thus depend on organic chelators which Lindsay reports would maintain an adequate iron supply by diffusion and mass flow at concentrations as low as 10^{-8} M^{1,2}. Hydroxamate siderophores (HS), microbially produced, ferric-specific, iron transport molecules with stability constants³ as high as 10^{32} , may represent the chelators which maintain these soil iron concentrations. Such peptide derivatives were shown to control iron availability in aquatic ecosystems⁴, but little is known about their soil role beyond their function as microbial growth factors^{5,6}. We report here the occurrence of HS in aqueous extracts of a variety of soils in concentrations (10^{-7} M- 10^{-8} M after correction to 10% soil moisture) sufficient to affect plant nutrition^{1,2}.

Soil samples (0-10 cm deep) were collected from 57 sites representing numerous biomes and soil types. After extraction (1:1 H₂O:soil, w/w) at 4 °C for 1 h, samples were centrifuged, the supernatants dried (90 °C), and the residues rehydrated, filter sterilized, and bioassayed using the HS auxotrophic bacterium *Arthrobacter flavescens* JG-9. The initial survey used a diffusion plate method developed by P.J.S. as described by Estep *et al.*⁷. Nineteen survey soils, and other experimental

materials, were assayed by a more sensitive turbidimetric, dilution-tube modification of the plate method. The mean values of quadruplicate samples were expressed as molar equivalents of the HS standard, desferrioxamine methanesulphonate (DFOM).

The specificity of any bioassay of complex natural substrates must be regarded cautiously. To verify the specificity of the *Arthrobacter*⁸ we tested the natural chelators, citric acid, oxalic acid, 2,3-dihydroxybenzoic acid and the catechol siderophore, enterochelin, at concentrations of 4×10^{-3} M to 4×10^{-10} M. No growth stimulation was detected. A suspension of haemin was weakly stimulatory⁹ in our assay and measured 7×10^{-9} M DFOM. Although the concentration of haemin in soil extracts is probably reduced during sample processing, stimulation by haemin or unknown compounds remains a possibility. The presence of secondary hydroxamates in highly concentrated soil extracts was demonstrated qualitatively by a positive Csaky test¹⁰.

Hydroxamate siderophore concentrations in excess of 3×10^{-9} M DFOM equiv. were found in the extracts of all 57 preliminary survey soils. These soils were from grasslands, areas of mixed herbaceous vegetation, and coniferous and deciduous forests across the US. In extracts of 19 soils, representing a wide range of pH, diethylenetriaminepentaacetic acid-extractable iron (DTPA-Fe) and organic matter content, HS concentrations ranged from 3.4×10^{-8} to 2.7×10^{-9} M DFOM equiv. (mean, 1.2×10^{-8} M; median, 8.5×10^{-9} M). No simple relation between HS levels and either soil pH or DTPA-Fe was evident. However, HS concentration was directly correlated with per cent organic matter ($r = 0.868$, $n = 19$).

Significant adsorption of HS to soil surfaces was indicated by the low recovery (range, 0.3-17.3%; mean, 3.7%) of the model compound DFOM added at 1 µg per g dry wt of soil. Proof of a large reservoir of natural siderophores came from repeated extractions of an unamended soil sample (Fig. 1). The early extractions yielded nearly the same quantity of HS (~ 0.03 nmol g⁻¹), but yield subsequently declined to about 0.01 nmol g⁻¹ per extraction. Total yield after 11 extractions was 0.18 nmol g⁻¹. DFOM added to a similar soil sample was more readily desorbed than was natural HS (Fig. 1). The decreasing yield of natural siderophores suggests that chemisorption on organic matter or migration to micropore sites occurs with time.

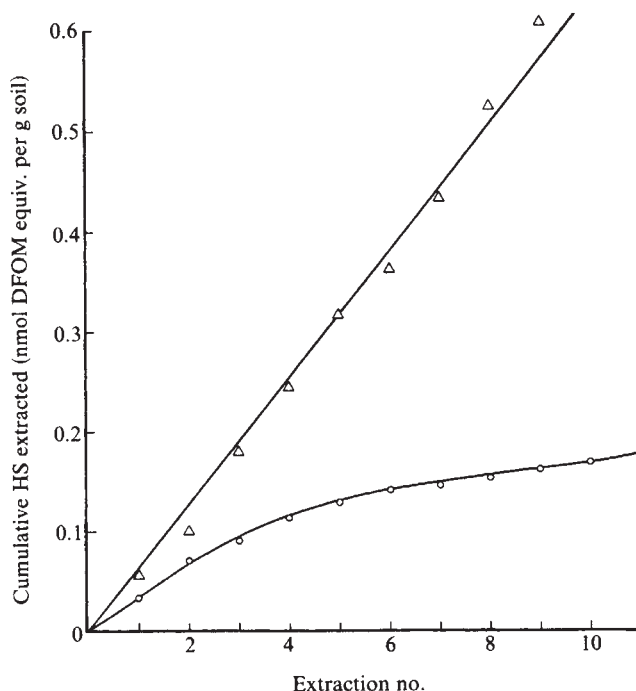


Fig. 1 Siderophore desorption (nmol per g soil) from soil (TC1) by the repeated extraction of amended DFOM at 1 µg per g dry wt soil (Δ) and natural HS (○).

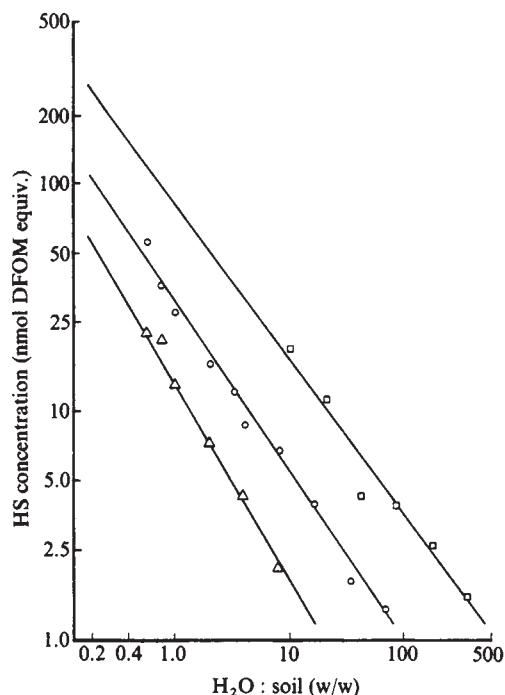


Fig. 2 Concentration (nmol) of natural HS in aqueous extracts of three soils at various H_2O :soil (w/w) ratios. (Δ), Soil TC9 with 2% organic matter, $1n y = 1n 13.5 - 0.873 1n x$; ($\circ$), soil TC1C with 10% organic matter, $1n y = 1n 29.8 - 0.760 1n x$; ($\square$), Canadian sphagnum peat with 95% organic matter, $1n y = 1n 80.2 - 0.686 1n x$.

To estimate the solution concentrations of HS in realistic soil moisture conditions, we extracted several soils at various H_2O :soil ratios. Siderophore concentration in solution increased markedly as soil moisture decreased (Fig. 2) and was clearly a power function of per cent soil moisture. Slopes of the curves were inversely correlated with soil organic matter content, as would be expected if the chelators were adsorbed to soil organic matter. A conservative estimate of minimum siderophore concentrations at 10% soil moisture was extrapolated from the value for this 1:1 H_2O :soil extract using the slope value from sphagnum peat containing 95% organic matter (see Fig. 2 legend). Extrapolated values for 10% moisture varied from 1.7×10^{-7} M to 1.3×10^{-8} M DFOM equiv., entirely within the range theoretically necessary to maintain an adequate iron supply to roots^{1,2}.

The ecological significance of hydroxamate siderophores in ferric iron mobilization is apparent when their stability constants (for example, ferrioxamine B, $\log K = 31$)³ are compared with those of the less specific organic acids (for example, ferric citrate, $\log K = 12$)¹¹, phenolates (for example, ferric *p*-hydroxybenzoate, $\log K = 15$)¹¹ and several synthetic chelators. Although the synthetic chelate ethylenediaminetetraacetic acid (EDTA) has a $\log K$ of 26 for ferric iron, it cannot effectively retain iron in soil solutions above pH 6 because of competition from other cations².

We have shown that hydroxamate siderophores are present in a wide variety of soils in concentrations that significantly increase the availability of iron to plants. We have also presented evidence that large reservoirs of these compounds are adsorbed to soil organic matter. We believe that HS in soils perform many chelate functions previously attributed to organic acids and polyphenolic humic material.

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Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport

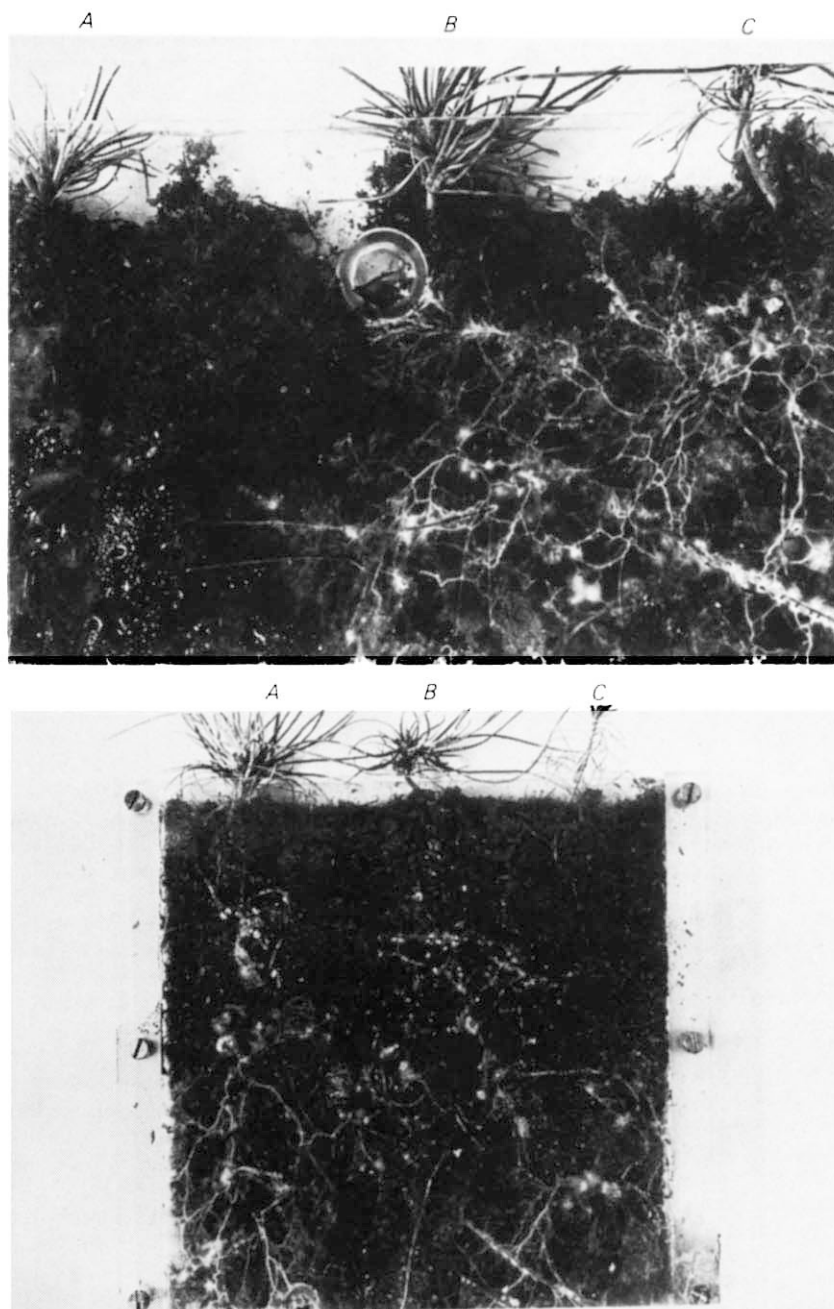
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Anastomosing systems of fungal rhizomorphs are found in association with mycorrhizal roots of trees in arid zones¹, in soils of temperate deciduous², coniferous³ and humid tropical⁴ forests, and even in mine spoils⁵. Although it is recognized that the fungus depends on the host for its carbon supply^{6,7}, the functional relationship between the partners has not been investigated fully. Transport of ³²P through rhizomorphs has been demonstrated⁸, but their potential to absorb and transport water is not known. We have synthesized mycorrhizal rhizomorphs in association with pine, and preliminary experiments with tritiated water (³H₂O) showed that the cut distal ends could absorb water which was transported to mycorrhizal roots and to seedling needles⁹. We have since developed a method for feeding ³H₂O to the intact terminal portions of rhizomorphs in sealed vessels with negligible disturbance to the mycelial system, and we report here that mycorrhizal rhizomorphs can absorb water and facilitate its transport over ecologically significant distances. Electron microscopy shows that the rhizomorphs are differentiated structures with large central 'vessel' hyphae.

Mycorrhizas were synthesized in aseptically conditions in 250-ml Erlenmeyer flasks which contained a peat-vermiculite mixture (1:4 v/v) moistened with modified Melin-Norkrans (MMN) solution. The medium was inoculated with a disk of *Suillus bovinus* mycelium. After the fungus had become established, aseptically germinated seedlings of *Pinus sylvestris* were transferred to the flasks which were then maintained in a growth chamber. After mycorrhiza formation had been confirmed, seedlings were transferred to fresh non-sterile milled peat in Perspex chambers (15 × 17 × 1 cm) with detachable sides and base. Three seedlings were placed on the surface of the peat with most of their mycorrhizal roots visible on the upper surface. The wall was then replaced, the chambers were wrapped in aluminium foil to exclude light and replaced in the growth cabinet. Within 142 d the peat was extensively exploited by rhizomorphs, many of which formed on the wall of the chamber. A shallow Perspex cup (1 cm in diameter, 1 mm deep), its rim coated with anhydrous lanolin, and containing 4 μ Ci of ³H₂O in 50 μ l water, was attached to the lower surface of the chamber wall enclosing the terminal parts of a rhizomorph system. Cups were placed either near the upper peat surface and hence in close proximity to the seedlings, or at the base of the chamber at least 15 cm from the seedling needles (Fig. 1). After exposure to the isotope for 1 h the rhizomorphs emerging from the cup were cut, dissected away from the wall and peat, and traced backwards to mycorrhizal roots. They were then cut into 5-mm segments, which were placed in sealed scintillation vials

Fig. 1 *a*, Upper part of root chamber with source cup containing $^3\text{H}_2\text{O}$ attached by means of anhydrous lanolin to terminal parts of rhizomorph system. Position of seedlings *A*, *B* and *C* are shown relative to source (Table 1*a*). *b* Shows position of attachment of lower source cup and of seedlings analysed (Table 1*b*).



containing 1 ml of NCS tissue solubilizer (Amersham/Serle). These, together with vials containing peat, root and needle samples, were incubated at 50 °C for 48 h, cooled to room temperature, and hydrolysed with 0.4 ml of 5N HCl before radioactivity was determined by scintillation counting. Similar rhizomorphs from the chambers and from a pine plantation were cut into 2–3-mm lengths, fixed and processed for examination by transmission electron microscopy.

Within 1 h, $^3\text{H}_2\text{O}$ moved from both the upper (Table 1*a*) and lower (Table 1*b*) source cups, through rhizomorphs to mycorrhizal roots and shoots. In each case movement occurred to more than one of the seedlings in the chamber, indicating that, as would be expected from visual inspection of the rhizomorph system (Fig. 1), the route was irregular. Beyond the root, passage of label preferentially to young needles (plants *A* and *B*, Table 1*a*; plant *A*, Table 1*b*) suggests a polarity of movement along recently laid down conduits in the seedling. Seedling *A* (Fig. 1*a*), to which no rhizomorphs were attached, contained no label, demonstrating that water movement was dependent on the presence of a rhizomorph connection from source cup to seedling. Detection of counts in needles up to 27 cm from the

source (Table 1*b*) indicates that a minimum rate of transport of 27 cm h^{-1} was obtained in these experimental circumstances. Such rates of transport, which are comparable with those frequently reported for xylem, suggest that the hydraulic conductivity of the pathway is high. It is likely that water moves through the rhizomorph along a gradient of water potential established by transpiration from the seedling.

Ultrastructural analysis reveals considerable differentiation in this type of rhizomorph. Transverse and longitudinal sections (Fig. 2) show that central hyphae of relatively large diameter, and which generally lack cytoplasmic contents, are surrounded by narrower densely cytoplasmic hyphae often with thick cell walls. These form a pseudoparenchymatous sheath around the central structures. Stages in the dissolution of transverse walls of the central hyphae, can be seen. At maturity these structures are analogous to the 'vessel' hyphae of *Serpula lacrymans*^{10,11}. Their diameters are between 6 and 20 μm which, together with the absence of cross walls and cytoplasm, makes them structurally comparable with xylem vessels of higher plants. This helps to explain the low resistance to water movement through the rhizomorphs revealed by the tracer studies, and lends support to

Table 1 Transport of water through rhizomorphs to mycorrhizal pine seedlings as revealed by liquid scintillation counting of $^3\text{H}_2\text{O}$ extracted from rhizomorph segments, mycorrhizal roots and needles

a, Source cup attached to wall at top of chamber (Fig. 1a)			b, Source cup attached to wall at base of chamber (Fig. 1b)		
Material sampled	Distance of material from source (mm)	Counts (d.p.m.)	Material sampled	Distance of material from source (mm)	Counts (d.p.m.)
Rhizomorph segment (0.5 cm)	10	3,231	Rhizomorph segments (0.5 cm)	10	1,476
	20	800		20	1,931
	30	2,060		30	1,019
	40	2,610		40	540
Mycorrhizal root attached to rhizomorph	45	2,338		50	689
Young (extending) needle of plant C	60–70	1,004		60	804
Old (fully extended) needle of plant C	50–60	0		70	913
Young needle of plant B	60–70	3,577	Mycorrhizal root attached to rhizomorph	80	43,452
Old needle of plant B	50–65	0	Lateral branch of rhizomorph	40	760
Young needle of plant A	70–80	0		50	851
Old needle of plant A	60–70	0	Mycorrhizal root attached to lateral branch	80	25,661
Peat below source	0	0	Young needle of plant A	170–190	13,761
			Old needle of plant A	160–170	458
			Young needle of plant B	230–240	2,091
			Old needle of plant B	180–220	1,641
			Young needle of plant C	240–270	984
			Old needle of plant C	200–230	0
			Peat below source	0	0

the view that water moves apoplastically through the central vessels rather than symplastically through the narrow outer hyphae or through the cell walls. The accumulation of activity in the sheath region may reflect increased resistance to flow in this area, where presumably a considerable portion of the pathway must be through the symplast. Certain of these structural fea-

tures would be expected to protect the vessel hyphae from collapse when under negative hydrostatic pressure. The parenchymatous sheath of the rhizomorph with its thick-walled members would supply external support, and the mycorrhizal sheath situated in the pathway between seedling and rhizomorph would supply a filtration resistance isolating the 'vessels' from the lowest seedling water potentials. The outer sheath of the rhizomorph also probably presents a high resistance to lateral movement of water which would explain the absence of isotope leakage into the peat (Table 1a).

Plants such as pine which are normally ecto-mycorrhizal have a relatively low root density compared to grasses and most other herbaceous plants^{12–14}. Our observations indicate that the rhizomorphs of ecto-mycorrhizas may represent functional extensions of the root system. We have now synthesized mycorrhizas from which rhizomorphs grow to exploit moist soil while the soil around the seedling roots is allowed to dry progressively. In these circumstances seedling water balance has been maintained for several weeks by means of water transport through the rhizomorphs. During the same period control seedlings lacking rhizomorphs died.

Mycorrhizal fungi differ in their capacity to produce rhizomorphs and there may be structural and functional distinctions between rhizomorphs produced by different fungal symbionts. Such features are now being investigated with a view to selection of mycorrhizal associates which have the capacity to improve the performance of forest crops particularly in semi-arid areas.

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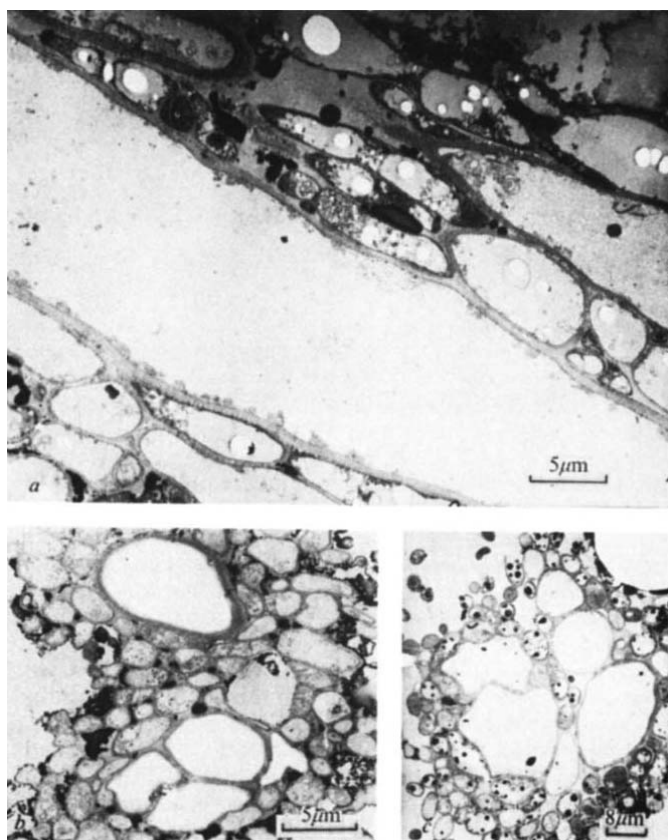


Fig. 2 a, Longitudinal section of rhizomorph from synthesized *Pinus sylvestris*–*Suillus bovinus* system. b, Transverse section of rhizomorph from the same system. c, Transverse section of rhizomorph attached to mycorrhizal root of *Pinus sylvestris* in the field. All tissues were fixed for 1.5 h in 2.5% phosphate-buffered glutaraldehyde, dehydrated in graded ethanol series and embedded in Spurr's resin.

Maragheh: a classical late Miocene vertebrate locality in northwestern Iran

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The fossil vertebrate localities of the Maragheh region in Azerbaijan, northwestern Iran, are among the most renowned late Miocene localities in Eurasia. The fossiliferous beds are found to the north and east of the town of Maragheh on the southern and eastern slopes of the Sahand massif and at an altitude of 1,450–1,900 m (Fig. 1). The first collection was made by Khanykof in 1840. Subsequent investigators of the fauna^{1–15}, established Maragheh as the site of one of the principal 'Pontian' mammal faunas of Eurasia. Recently, Tobien¹⁶, Erdbrink *et al.*¹⁷, Takai *et al.*¹⁸ and ourselves (the Lake Rezaieyeh Expedition) have concentrated on developing the geochronological and palaeoenvironmental aspects of the Maragheh fauna. The similarity of the Maragheh fauna to those of Samos, Pikermi, Saloniki and Mt Luberon, in particular, was recognized by most workers. The most significant results of the investigations reported here are that the Maragheh fauna spans an extended geochronological range, ~9.5–7 Myr, that the so-called 'Pontian' mammal communities are provincially distributed, and diachronic in their appearance, and finally that the absence of hominoid primates from this site and other late Miocene sites with similar savannah-like faunas strongly suggests their adaptation at this time to more forested environments which existed in Eurasia and Africa before and during the Maragheh interval.

The late Miocene deposits in the Maragheh Basin consist of a thick series of volcanoclastic and pyroclastic continental strata which underlie a broad pediplain on the southern and eastern sides of the Sahand volcanic massif. On the southern side of Sahand the strata are virtually undeformed, and very gently inclined to the west-southwest. The entire late Miocene section has previously been termed the Maragheh Formation¹⁹, but we restrict this name to the volcanoclastic series, and distinguish a basal pyroclastic formation, the Basal Tuff, on lithological and stratigraphical grounds. South of Sahand the Maragheh Formation is eroded to a thickness of about 300 m and consists of strata made up exclusively of detrital fragments of hornblende, andesite lava and pumice, interbedded at widely spaced intervals with layers of pumice-lapilli tuff. These nominally andesitic beds are generally unlaminate, poorly sorted silty grits with cobble lenses of andesite and hard iron-stained pumice cobbles, in depositional units 1–3 m thick. The siltstone beds are the most fossiliferous and consist of massive poorly sorted volcanoclastic overbank deposits 3–10 m thick. We interpret each of the fining-upwards sequences as having been produced by a single flood event, or perhaps waxing and waning events within a single flood. The amount of sediment laid down in any particular flood is thus variable, but no major hiatuses have been found in the stratigraphic succession.

The interbedded pumice-lapilli tuffs are composed of soft, angular blocks and chips of snow-white andesitic pumice mixed to a greater or lesser extent with andesitic lava detritus. Laminated, upper parts of the pumice-lapilli tuffs obviously were reworked to a certain extent, but the basal pumice zones are 'draped' over palaeotopographical relief (buried gullies) with constant thickness, indicating primary pyroclastic deposition.

The Basal Tuff is (apparently) a single airfall unit of rhyolite tuff with a local thickness of over 80 m. The unit is a uniform, unbedded, structureless deposit of white, devitrified ash with many randomly orientated flakes of mica and fresh crystal fragments of feldspar and quartz. The Maragheh Formation seems to rest on the Basal Tuff with a low-angle regional unconformity. The stratigraphical data, supported by the radiometric and biostratigraphical evidence, indicate that the

Table 1 Isotopic age determinations from the Basal Tuff and Maragheh Formation

Distance from LC (m)	Stratigraphic level	Amini (this work) Zircon FT	Drake (this work) Total-fusion K-Ar	Kamei <i>et al.</i> ¹⁹ Zircon FT	Erdbrink <i>et al.</i> ¹⁸ Total-fusion K-Ar
+100	Korde-deh pumice			5.2	730-04
	Murdaq Chai				
+90 est.	Upper pumice	7.2±0.4 R10	7.5±0.4 R10pl		
	Il'kchi				
+60 est.	Middle pumice	7.3±0.5 R5	7.7±0.5 R5pl		
	Il'kchi	(R6)	7.2±0.5 R6pl		
+15 est.	Lower pumice	7.4±0.4/ R8/	7.9±0.7 R8pl		
	Il'kchi	7.0±0.4 R8			
+(15) est.	Village pumice	5.2±0.3/ R9/	5.5±1.0 R9pl		
	Il'kchi	5.3±0.3 R9			
+7	Layered marker pumice	7.6±0.5 R2	8.3±0.9 R2pl		8.1±0.4 VIIWR
	Scholl'avand				6.2±0.6 VIIhb
		7.7±0.5 R12	6.4±0.6 R12pl		
			6.9±0.3 M5hb		
	Loose chippings pumice	7.8±0.4 R11	7.4±0.3 R11pl	6.6	730-19
	Scholl'avand				
-50	Gürt Dareseh pumice			6.5	730-17
	Murdaq				14.4±0.7 VWR
-110	Ignimbritic tuff	10.6±0.8 R3	8.8±0.5 R3pl	7.0	730-10
	Murdaq		8.9±0.5		12.9±0.7 IWR
	Basal Tuff		11.2±0.5 M1bi	7.2	730-20
	Western area				10.1±0.5 IXWR
	Basal Tuff	11.2±0.6 R4	10.1±0.5 R4bi		11.4±0.5 IXbi
	Il'kchi		9.3±0.1 R4an		
		12.8±0.5 R7	11.3±1.0 R7bi		
			9.3±0.1 R7an		

All fission-track ages are derived from separated zircon crystals. Total-fusion K-Ar age determinations were obtained from materials indicated as follows: pl, high-temperature plagioclase; hb, hornblende; bi, biotite; an, high-temperature anorthoclase; WR, whole-rock samples. Decay constants $\lambda\beta = 4.962 \times 10^{-10} \text{ yr}^{-1}$, $\lambda\epsilon = 0.581 \times 10^{-10} \text{ yr}^{-1}$; isotopic abundance: $^{40}\text{K} = 0.01167\%$ of total K. Decay constants and isotopic abundance used by Erdbrink *et al.*¹⁸ according to pre-1978 standards are slightly different, but do not change the computed values significantly. Kamei *et al.*¹⁹ do not give their precision limits but state (p. 157) that these are "usually under 10%". Analytical details of new dating presented here are available from M.H.A. and R.D.

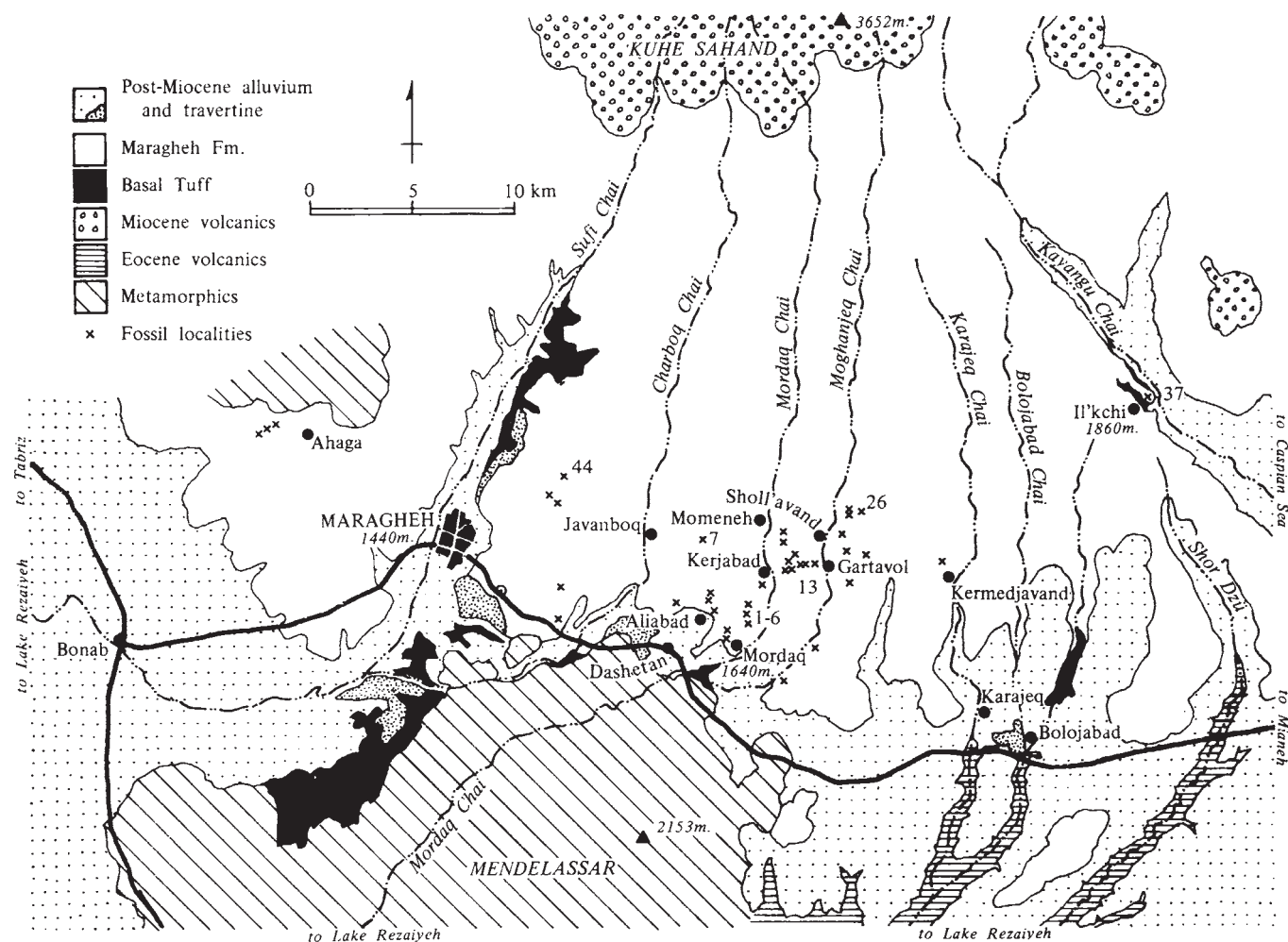


Fig. 1 Map of the southern part of the Maragheh Formation.

beds farthest to the west, and the fossils which they contain (at Kopran) were the earliest to have been deposited, and Il'kchi, the easternmost locality, seems to correlate with the youngest localities in the central area although it lies directly on the Basal Tuff. In the fossiliferous central area, the 'loose chippings' marker bed (LC), a distinctive and easily traced volcanoclastic breccia, was used as the key horizon for the allocation of Maragheh fossil localities to arbitrary 25-m stratigraphical intervals. The marker bed was also mapped over part of its exposures by Kamei¹⁹ as the 'Scoria Bed', and it appears at the top of two of the sections measured by Erdbrink *et al.*¹⁸ as 'trachytic breccia'.

We report here on 28 age determinations from the Basal Tuff and the Maragheh Formation in the fossiliferous district east of Maragheh. As summarized in Table 1 and Fig. 2, these determinations are based on 3 samples of the Basal Tuff and 10 samples from pyroclastic layers in the Maragheh Formation. Four of the five horizons dated by Erdbrink *et al.*¹⁸, and three of the five horizons dated by Kamei *et al.*¹⁹ from the same late Miocene sequence are represented in our samples. Our geochronology and that of Kamei *et al.*¹⁹ are consistent with the stratigraphy described here, but the Kamei ages are systematically younger than ours at the same levels. The ages reported by Erdbrink *et al.*¹⁸ on Maragheh pumicites are slightly to significantly older than ours from the same horizons, and are also inconsistent with the present stratigraphy, whereas their age analyses of the Basal Tuff are comparable with ours (see below).

As shown in Table 1, 11 of the 14 samples were dated both by fission-track (FT) analysis of pyroclastic zircons (M.H.A.) and by total-fusion K-Ar analysis of separated minerals (R.D.). Both biotite and anorthoclase were K/Ar dated in two Basal Tuff K-Ar samples. Of the 28 age determinations obtained, only one pair of FT and K-Ar ages (sample R3) were significantly different, and only one sample (R9) had an age that was clearly inconsistent with a simple superpositional interpretation. Furthermore, three horizons—Il'kchi middle pumice, layered marker pumice and Basal Tuff—were dated in at least two different places, with good to moderate agreement.

The abundance of data, and the quality of the stratigraphy, nevertheless, clearly indicate problems in Maragheh geochronology that would not be apparent if there were only a few age determinations. There can be little question that the upper level of fossiliferous beds in the central area, and the isolated Il'kchi locality which we have correlated with this level, should be calibrated by the averages of 7.4 Myr respectively obtained on the directly superjacent pumicites in both areas. Unfortunately, the age determinations from the lower part of the section in the central area (for example, the ages obtained from the Murdaq 'ignimbrite' and the Gürt Dareseh pumicite) seem to be unreliable due to their large variation, and in addition two interpretations are possible from the dating of the Basal Tuff. We prefer to accept an age of 8.9 ± 0.5 Myr for the Murdaq ignimbrite as being both analytically satisfactory and stratigraphically consistent, the other, contradictory dates notwith-

standing. We also conclude that the oldest Maragheh fossils are virtually certain to be younger than the cluster of FT and biotite K-Ar ages from the Basal Tuff, ~11 Myr, and may be younger than the age of the anorthoclase K-Ar age determinations of 9.3 Myr.

All the pyroclastic units in the Maragheh Formation contain accidental pebbles and grains of detrital andesite. For this reason, whole-rock age determinations on such units¹⁸ must be regarded as dubious. Only the fact that the accidental contaminants are predominantly if not entirely derived from outcrops on the Miocene Sahand volcano, and not from even older rocks, gives such determinations an appearance of reliability. The Basal Tuff seems to contain no accidental fragments; on the other hand, whole-rock analysis would include the altered and devitrified matrix, from which some argon may have been lost. The discordantly young age reported by Erdbrink *et al.*¹⁸ on a whole-rock sample may be erroneous for this reason. The lack of agreement between FT ages and anorthoclase K-Ar ages, as well as the discrepancy between biotite and anorthoclase K-Ar ages from the Basal Tuff is now under investigation.

The dating reported here gives a relatively unambiguous age for the upper limit of the Maragheh fossiliferous sequence of 7.4 Myr; preliminary and somewhat equivocal interpretations of the Basal Tuff samples suggest a maximum lower age limit of 11 Myr, but probably not more than 10 Myr. These limits are not limits of certainty, but represent the actual time-span of the Maragheh fossil fauna, interpreted here to be from about 9.5 to 7 Myr.

The biostratigraphy presented here (Table 2) combines the fossil mammal collections made by the Lake Rezaieyeh Expedition and Tobien's excavations, with information reported by

Erdbrink *et al.*¹⁸, Kamei *et al.*¹⁹ and Bernor²⁰. The Maragheh mammalian fauna is divided into three biostratigraphic intervals: lower Maragheh, extending from -150 m to -50 m below LC; middle Maragheh, extending from -50 m to -25 m below LC; and upper Maragheh, extending from -25 m to +25 m below/above LC.

In addition to the radiometric determinations cited above, detailed work on circum-Mediterranean East African late Miocene *Hipparion* evolution²¹ has evaluated a recent analysis of *Hipparion* superspecific groups for use in provincial correlations between the Maragheh sites and several other Old World localities. These interpretations suggest that the Lower Maragheh *Hipparion* sp. 3 has a more primitive facial morphology than any of the *Hipparion* species from the Greek localities but compare closely with the early Vallesian *Hipparion* species *Hipparion catalaunicum*²² and *Hipparion africanum*²³. The Middle Maragheh *Hipparion* *H. cf. prostylum* occurs at Pikerimi, Saloniki and Mt Luberon²⁴, and *Hipparion cf. mediterraneum* occurs at Pikerimi, suggesting correlation of these localities with this Maragheh interval. In the Upper Maragheh, *H. cf. Hipparion dietrichi*, *Hipparion mathewi* and *H. cf. H. mediterraneum* are morphologically the most derived species of *Hipparion* in the sequence. These taxa, as well as *H. cf. Hipparion proboscideum* and *Hipparion 'minus'*, occur at Samos within a restricted chronological range (~0.5 Myr; N. Solounias, personal communication), suggesting correlation of Samos with Upper Maragheh, despite dating that indicates a relatively older age for the Samos material²⁵.

The biochronological interpretation of the Maragheh sequence given here broadly supports the radiometric determinations that refer it to an early Turolian to medial Turolian

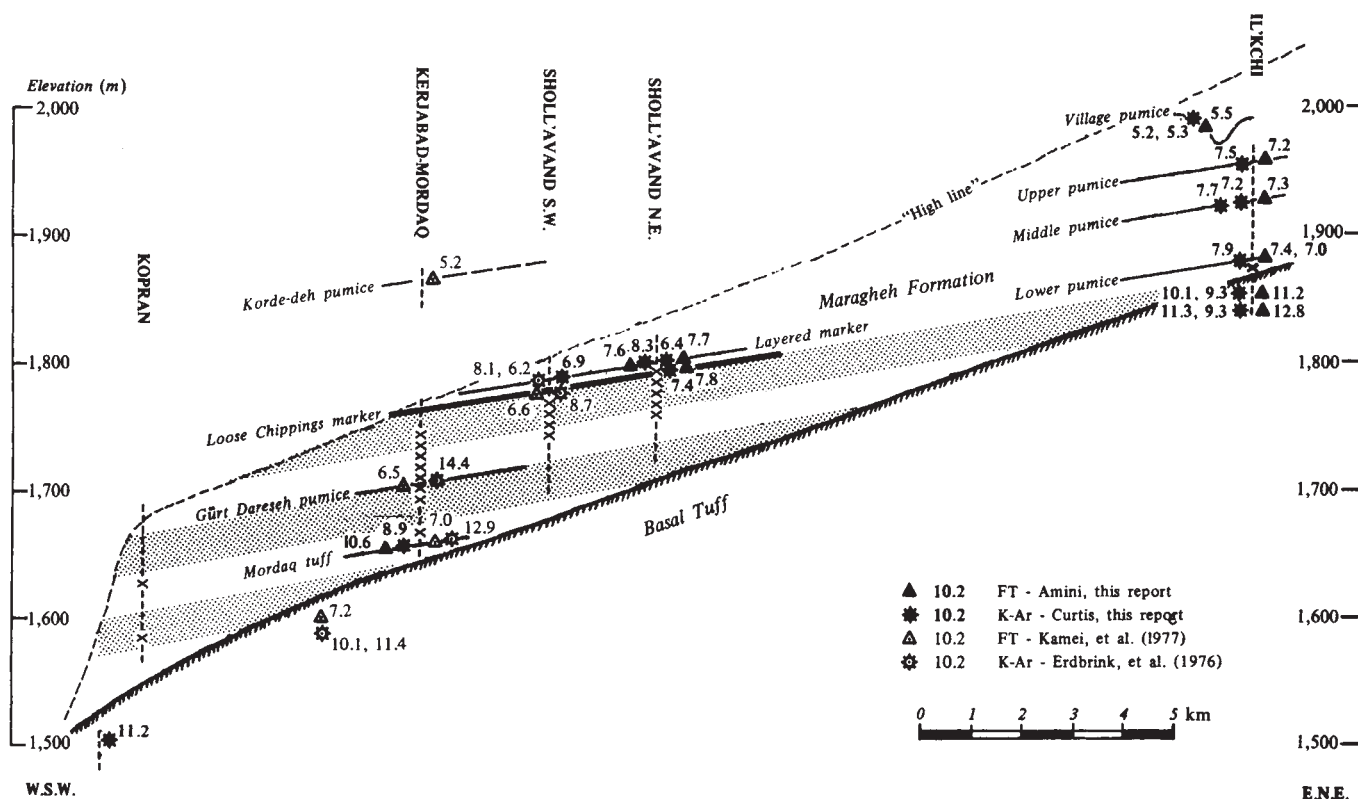


Fig. 2 Stratigraphical display of isotopic age determinations from the Maragheh Formation and Basal Tuff. See Table 1 for sample documentation. The FT and K-Ar ages obtained by Amini and Drake from the same samples are shown adjacent to one another. Repeat determinations, or determinations made on different materials from the same sample, are separated by commas. The sampling profiles on the line of section are generalized indications of the measured and studied thickness in the respective areas; portions shown by 'x' symbols represent intervals containing abundant mammalian fossils. Biostratigraphic subdivisions in 25-m increments from the plane of the LC marker are indicated by the stippled pattern. The 'high line' or interfluvial maximum elevation is the residual thickness of the Maragheh Formation along the line of section inferred from the highest level of terrace deposits; thickness increases towards Sahand, where the Korde-deh tuff crops out.

Table 2 Stratigraphical ranges of Maragheh mammalian taxa

Distance above/below LC (m)	Stratigraphical Position of MMTT localities	
+25		
0	39 26 37 39	
-25	24 32 38 22 16 47 27 29 15 49 49A 25 50 31 33 13 40 51 21	<i>Mesopithecus pentelici</i> <i>Martes</i> sp. indet. <i>Promeles palaeattica</i> <i>Melodon maraghanus</i> <i>Parataxidea polaki</i> <i>Machairodus aphanistus</i> <i>Orycteropus</i> cf. <i>gaudryi</i> <i>Choerolophodon pentelici</i>
-50	18 7 14 6 19 20 34 1C 1B 2 11 4 17 35 3	<i>Deinotherium</i> sp. indet. <i>Hipparion</i> cf. <i>prostylum</i> <i>Hipparion dietrichi</i> <i>Hipparion matthewi</i> <i>Hipparion mediterraneum</i> <i>Hipparion</i> cf. <i>proboscideum</i> <i>Ancylotherium pentelici</i> <i>Microstonyx erymanthus</i> Cervidae gen. and sp. indet. Giraffidae gen. and sp. indet. <i>Palaeotragus coelophrys</i> <i>Samotherium</i> sp. indet. <i>Miotragocerus rugosifrons</i>
-75	1A 5 8 42 45 23	<i>Chilotherium persiae</i> <i>Diceros neumayri</i> <i>Gazella rodleri</i> <i>Prostrepsiceros houtumshindleri</i> <i>Protragelaphus skouzesi</i> <i>Oioceros atropames</i> <i>Oioceros rothi</i>
-100	36 28	<i>Chilotherium persiae</i> <i>Diceros neumayri</i> <i>Gazella rodleri</i> <i>Prostrepsiceros houtumshindleri</i> <i>Protragelaphus skouzesi</i> <i>Oioceros atropames</i> <i>Oioceros rothi</i>
-125	44	<i>Chilotherium persiae</i> <i>Diceros neumayri</i> <i>Gazella rodleri</i> <i>Prostrepsiceros houtumshindleri</i> <i>Protragelaphus skouzesi</i> <i>Oioceros atropames</i> <i>Oioceros rothi</i>
-150	41 43 48	<i>Chilotherium persiae</i> <i>Diceros neumayri</i> <i>Gazella rodleri</i> <i>Prostrepsiceros houtumshindleri</i> <i>Protragelaphus skouzesi</i> <i>Oioceros atropames</i> <i>Oioceros rothi</i>

BASAL TUFF

age (MN 11 and 12, ref. 26). Pro-rated ages for the various Maragheh biostratigraphical intervals can be advanced here as: ~9.5–8.5 Myr for Lower Maragheh; 8.5–8.0 Myr for Middle Maragheh; 8.0–7.5 Myr for Upper Maragheh.

No complete, articulated skeletons were found. Taphonomic studies in a single quarry, MMTT-13, revealed 48% of the bones to be part of smaller, articulated units, predominantly distal limb segments. Skulls with mandibles and vertebral strings were also present, fragmented single bones and isolated teeth rare, complete or almost complete disarticulated limb bones and mandibular rami common. The bones showed no preferred orientation; few were of animals estimated to have weighed less than 10 kg. Taphonomic data support the interpretation that the fossils were incorporated in overbank deposits and either buried almost immediately or exposed long enough to allow removal of only a few elements by scavengers.

The Maragheh fauna has long been thought to be one of the typical Turolian savannah faunas of Eurasia. Preliminary studies of habitat spectra²⁷ suggest that the ecological diversity spectra are not typical of modern savannah, which is dominated by wooded grassland habitat, but are what would be expected in a savannah dominated by woodland with adjacent wooded grassland.

As shown in the East African record, the hominids did not join the savannah biome until the Pliocene, and the Maragheh ecological setting is too open and seasonal for the dry-opithecines, sivapithecines (and related forms such as *Ramapithecus*)²⁸. The presence of a single *Mesopithecus* from Maragheh demonstrates that these animals were in the vicinity even though the predominant fauna was probably derived from the more open woodland and wooded grassland habitat.

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Embryogenesis in a *Drosophila* mutant expressing half the normal segment number

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Insect segments are seriated developmental subunits whose borders largely control intra-segmental patterning¹. The individual segment owes its specific character to position-dependent instructions given to its early embryonic progenitor cells². In *Drosophila*, the segment borders first become visible as a series of transverse infoldings on the surface of the germ band³. We describe here development in a mutant (*prd^{FR1}*) which forms half the normal number of transverse infoldings spaced at twice the normal distance, thereby subdividing the germ band into a series of giant domains (Fig. 1 a–c). Each domain develops the tracheal pits of two normal segments (Fig. 1e), thus revealing its composite origin, but the larva expresses only one segment border per domain and the cuticle between these borders is similar to the normal segment in both size and zonation (Fig. 2). We conclude that this course of development reflects a conflict between the seriated instructions specifying local segment character (see Fig. 1b) and controlling influences exerted by the abnormally spaced infoldings and later by the definitive segment borders.

The mutant we describe maps at 2.43 ± 0.7. It was obtained by EMS treatment of wild-type males with ethyl methane sulphonate and subsequently found to be allelic to the *paired* mutants⁴; the allele, which we designate as *prd^{FR1}*, is balanced over the CyO chromosome. In approximately 25% of the eggs, probably the homozygous fraction, gastrulation seems to be slightly delayed and the cephalic furrow either fails to appear or forms some time after the onset of germ-band extension. The maximum length of the extended germ band exceeds 90% of the normal value. When transverse epidermal furrows first become visible in the scanning electron microscope (Fig. 1), they are spaced at about twice the normal distance, demarcating six giant domains, each of which contains about as many cells as two segment anlagen in the same region of the normal germ band. The posterior domains develop tracheal pits in a rather peculiar way (Fig. 1 d–f). In wild-type embryos, the corresponding segments each form a single pair of pits which subsequently disappear in the anterior border furrow⁵. In the mutant, one pair of pits forms next to each furrow and slightly later another pair develops more posteriorly. The anterior pits then disappear, as occurs in normal development, but the posterior pits persist on the body flanks, and the ensuing tracheal system is severely disrupted. Within or just behind the anteriormost epidermal infolding, a pair of invaginations form (Fig. 1e, f); we consider these to be the anlagen for the salivary duct because they later fuse mid-ventrally as in wild type embryos³. After head involution the ventral rim of the pre-oral cavity produces the larval cuticle markers⁶ characterizing the anterior border of the prothorax. The methods of observation used do not allow us to determine whether the transverse folds give rise to the definitive segment borders, as they do in normal development.

The larva moves in the egg case but fails to hatch. In its cuticle, segment length exceeds that of the wild type by a factor of 1.2–1.5. The cuticle of the oversized segments is zoned as in normal segments⁵ (Fig. 2) but each zone expresses traits characteristic of two consecutive segments (hence 'paired'⁴). The traits of the anterior partner of each segment pair are restricted to a narrow anterior strip of cuticle⁴. A comparable reduction of the traits of the anterior segments occurs in the head and the caudal



Fig. 1 Scanning electron micrographs of *Drosophila* embryos prepared by the technique of Turner and Mahowald³. *a, c*, Ventral views of normal-spaced and double-spaced patterns of epidermal infoldings at the stage of maximum germ band extension. Transverse lines link double-spaced infoldings in the mutant *paired* to the normal pattern. Embryo in *a* (possibly heterozygous for *paired*) is abnormal in showing a weaker expression of every second furrow (these are the furrows present in Fig. 1*c*). Lettering in *b* identifies segments in *a* but can also be taken to symbolize a prepattern specifying local segment character. Abbreviations:

md, mandibular; mx, maxillary; lb, labial segments (head); I–III, thoracic segments; 1–3, anterior abdominal segments. A slight backward shift of the infoldings (dashed lines) is indicated by some observations and might account for the suppression of homologous cuticle elements in every second segment of the normal pattern⁴. *d–f*, Lateral views of germ band stages, ventral side at the left (negatives *d* and *e* were reversed to obtain this orientation). *d*, Onset of germ band shortening, to show anterior tracheal pits (arrows). *e*, Slightly later stage, posterior tracheal pits apparent and in most pairs linked to the anterior pit by a groove (one pair marked by arrows at bottom). The foremost anterior pit (top arrow) is about to enter the transverse furrow. *f*, The germ band is further contracted, posterior pits (horizontal arrows) persist on body flanks (albeit shifted anteriorly) and anterior pits have disappeared. Arrows at the top mark salivary duct pits also seen in *e*. *c–f* Represent typical specimens selected from more than 100 germ band stage embryos studied with the scanning electron microscope. Length of preparations varies between 0.33 and 0.4 mm.

complex. Here the H-piece (anterior part of the cephalopharyngeal skeleton) and the supra-anal tuft are markedly reduced; the former is derived from the labial segment⁶, the latter from the rudimentary 9th abdominal segment³. The protuberances carrying the posterior spiracles and their atria (8th abdominal segment) are abnormally large and somewhat deformed, providing further evidence for the spatial prevalence of the posterior partner in the paired segments. Up to 45% of mutant embryos show holes in the larval cuticle and/or lateral (rarely ventral) fusion of adjacent denticle belts. No large-scale cell elimination which could account for these anomalies or for the reduction in segment number or segment size was noted.

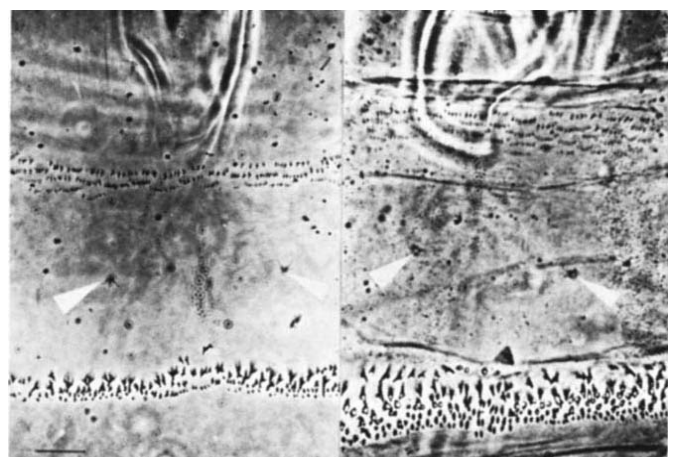
The abnormal segmentation pattern in *paired* is not linked to a specific local segment character. This was shown by combining the mutant with an extreme *Ultrabithorax* allele which confers mesothoracic character on all segments between the mesothorax and the posterior spiracles⁷. The ensuing larvae (if not disorganized) exhibit thoracic transformation of all abdomen-type segments but retain the abnormal distribution of the epidermal infoldings and the reduced number of definitive segment borders.

Abnormal subdivision of the germ anlage as described here is a novel type of developmental anomaly. We propose to name it 'embryonic malpartitioning'. We assume that the mutation primarily affects the positioning of the transverse ectodermal infoldings thought to mark the boundaries between adjacent segments³. The resultant abnormal pattern of furrows (Fig. 1*c*) seems to act as an aberrant 'partitioning grid' imposed on a germ anlage which carries the normal set of instructions for individual segment character (shown diagrammatically in Fig. 1*b*). This view is based on the findings that (1) the incipient spatial pattern of transverse furrows is more abnormal than the pattern of other visible epidermal Anlagen (tracheal and salivary duct pits, Fig. 1), and (2) the mutant generally maintains the normal spatial proportions of abdomen and thorax in the germ band and larval cuticle. In any case, the abnormal subdivision of the germ band must progressively influence subsequent patterning, because the tracheal pits appear in full number and nearly normal spacing (Fig. 1*e*) whereas the cuticle which forms later shows only half the normal number of segments. Despite their composite character these segments approach normal segment length, although not as closely as do the fused segments observed in the bug *Oncopeltus*¹.

Why the mutant should so exactly double-space the grid of epidermal infoldings (Fig. 1*c*) is a most intriguing question. To

assume suppression of every second furrow of the normal pattern merely alters the problem because some wild-type patterned germ bands from our mutant stock partly suppress the alternative set of furrows (Fig. 1*a*). The double-spacing therefore probably reflects some basic property of the mechanisms responsible for the periodic positioning of epidermal infoldings in normal development. Mechanisms based on dissipative structures which can be envisaged as a spatial series of 'stationary waves'^{8,9} could be inherently prone to this type of partitioning error; for instance, the mutant cells might recognize only the positive or the negative maxima (or zero transitions) whereas the wild-type cells could respond to both (possibly in succession). However, abnormal distribution of the periodic signals themselves cannot be excluded. Early anomalies like the defective cephalic furrow and subnormal germ band length might indicate changes in the geometrical boundary conditions which are critical for dissipative patterning and probably also for other spacing mechanisms. Whether the epidermal infoldings in the mutant initiate the full sequence of functions characterizing

Fig. 2 Phase contrast micrographs of ventral cuticle from the posterior thoracic region of *paired* (right) and wild-type (left) first instar larva. Note the typical transverse zonation of the mutant segment, and the increase in the number of denticle rows roughly proportional to the increase in segment length; a similar 'multiplication of elements'¹³ has been described previously in oversized segments¹⁴. Arrows mark Keilin's sense organs. The band of denticle rows at the bottom is the anterior rim of the abdomen. Scale bar, 0.02 mm.



a typical segment border (including its function as compartment boundary¹⁰) is not known. It even remains to be shown (for example, by defect mapping⁵) whether the infoldings in the mutant harbour the progenitors of those hypoderm cells which subsequently express the cuticular segment borders. In the absence of proof for this, we can consider alternative possibilities. The most attractive of these is that the folds form across the prospective territory of the anterior segment of each pair (Fig. 1b) and that in this abnormal position they prevent some region of the prospective hypoderm from expressing its strip of the cuticular pattern. If the starting conditions for periodic patterning were liable to several kinds of disturbance, the transverse folds might be shifted to different positions (the 'floating grid' hypothesis). The resulting seriated cuticular defects should then affect different strips of the intra-segmental pattern. Several classes of mutant exhibiting such defects have been found⁴ and observations on their early development may serve to test this assumption.

If, on the other hand, the infoldings represent functional

segment borders, their aberrant distribution raises the question of how in normal development the pattern of segment borders is kept in register with the pattern of instructions specifying local segment character (Fig. 1b). One possibility is that both patterns develop independently on the basis of the same axial system of positional information, for instance, an axial gradient^{2,11}. The mutant described here would then misinterpret this information when spacing its segment borders, but not when defining local segment character. Homoeotic mutants like *Ultrabithorax*, on the other hand, would misinterpret the same positional information with respect to local segment character^{11,12}. The former error would result in aberrantly demarcated segments, the latter in assigning a wrong character to some correctly demarcated segments.

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Parathyroid hormone stimulates bone resorption via a Na-Ca exchange mechanism

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Parathyroid hormone (PTH) stimulates the release of calcium from bone and is thought to initiate its action by a direct effect on the plasma membrane of bone cells¹. Although specific membrane receptors for PTH have not yet been identified in bone, they have been characterized in kidney², and PTH does stimulate production of cyclic AMP in bone³. However, the mechanism by which PTH causes the release of Ca from bone is not understood. We have determined that several agents that alter ion transport across biological membranes inhibit PTH-stimulated bone resorption *in vitro*. These agents include ouabain, veratridine and certain monovalent cation ionophores, which transport monovalent cations selectively^{4,5}. The inhibition of bone resorption is dose dependent and reversible within the first 24 h of treatment. All these compounds would be expected to increase intracellular sodium concentration in bone cells. Also, we find that decreasing the extracellular Na⁺ concentration prevents PTH-stimulated resorption. Therefore, we propose that PTH acts on bone to stimulate Ca release by means of a Na-Ca exchange mechanism. Decreasing the Na gradient across the bone cell plasma membrane would prevent the Na-Ca exchange and thus inhibit the physiological response to PTH.

PTH stimulates the release of Ca from bone in organ culture, an action analogous to its physiological effect on the skeleton *in vivo*¹. Studies in bone have demonstrated an early stimulated

increase in Ca influx both *in vivo*⁶ and *in vitro*⁷. A possible role for Na ions in hormonal stimulation of Ca release from bone has been postulated⁸ but not demonstrated experimentally.

Our results using intact neonatal mouse calvaria in organ culture show that monovalent cations do play an important role in the release of Ca from bone that is enhanced by PTH. Bone resorption stimulated by 0.1 U ml⁻¹ PTE (United States Pharmacopeia Parathyroid Extract, Eli Lilly Company) was inhibited by veratridine, ouabain and the monovalent cation ionophores, nigericin, monensin and X-206 (Table 1). Inhibition by ouabain was reversed almost completely by KCl (Table 1, Expt 2) whereas KCl had no effect on the inhibition produced by the monovalent ionophores (for example, Table 1, Expt 4).

Inhibition of stimulated bone resorption by nigericin was reversible within the first 24 h that the ionophore was present (Fig. 1a). However, after 48 h in the presence of both PTH and ionophore, the inhibition of bone resorption was no longer reversible (Fig. 1b). Similarly, if calvaria were pretreated with nigericin alone and then stimulated with PTE after washing out the ionophore, there was no subsequent response to PTE after prolonged (>24 h) pretreatment with ionophore. When similar experiments were performed with ouabain, the results were comparable; inhibition of stimulated resorption was reversible within the first 24 h of treatment, but not after 48 h of treatment with ouabain (N.S. K., unpublished observations).

To test directly for the role of monovalent cations in the PTH-stimulated Ca release from bone, experiments were performed in medium containing various concentrations of Na⁺ and K⁺ (Table 2). Decreasing the Na⁺ concentration below the control level of 150 mM resulted in a dose-dependent inhibition of PTE-stimulated Ca release from the calvaria. Below 100 mM Na⁺ there was no response to PTE. Removing K⁺ from the medium had no significant effect on PTE-stimulated bone resorption nor on the inhibition of stimulated resorption produced by ouabain or nigericin. However, increasing the K⁺ concentration above the control level of 5 mM blunted or reversed the inhibition by ouabain of PTE-stimulated resorption without altering the inhibition produced by nigericin.

All of the inhibitory agents used in this study alter monovalent ion fluxes across the plasma membrane and would be expected

Table 1 Inhibition of PTH-stimulated bone resorption *in vitro*: effects of veratridine, ouabain and monovalent cation ionophores

Expt no.	Treatment	Medium calcium (mg dl ⁻¹)
1	Control	6.2 ± 0.67
	PTE (0.1 U ml ⁻¹)	13.8*
	Veratridine (0.3 mM)	6.2
	PTE (0.1 U ml ⁻¹) + veratridine (0.3 mM)	6.3
2	Control	7.1 ± 0.88
	PTE (0.1 U ml ⁻¹)	12.8*
	KCl (15 mM)	8.0
	PTE (0.1 U ml ⁻¹) + ouabain (0.3 mM)	6.1
	PTE (0.1 U ml ⁻¹) + ouabain (0.3 mM) + KCl (15 mM)	10.4*
3	Control	8.0 ± 0.79
	PTE (0.1 U ml ⁻¹)	15.5*
	PTE (0.1 U ml ⁻¹) + nigericin (10 ⁻⁷ M)	7.5
	PTE (0.1 U ml ⁻¹) + monensin (3 × 10 ⁻⁷ M)	6.4†
	PTE (0.1 U ml ⁻¹) + X-206 (10 ⁻⁷ M)	8.1
4	Control	6.3 ± 0.46
	PTE (0.1 U ml ⁻¹)	9.4*
	KCl (15 mM)	7.2
	PTE (0.1 U ml ⁻¹) + nigericin (10 ⁻⁷ M)	6.0
	PTE (0.1 U ml ⁻¹) + nigericin (10 ⁻⁷ M) + KCl (15 mM)	5.7

Calvaria from 4–5-day-old neonatal mice were incubated at 37 °C in Dulbecco's modified Eagle's medium containing heat-inactivated (56 °C, 60 min) horse serum (15%) and fetal calf serum (2.5%)^{15,16}. Treatment was begun at zero time with concentrations of each agent which were determined to be maximally effective by complete dose-response experiments. Cultures were gassed daily and the medium changed once at 24 h. Medium Ca concentrations were measured at 24-h intervals using an automatic fluorometric titration method. Values given are the mean of at least four bones per group at the end of a 72-h treatment period. By analysis of variance the s.e. was calculated from the residual error term for each experiment. Because the number of bones in each group in a given experiment was the same, the s.e. for each group was the same, and therefore for simplicity the s.e. is given only for the control group in each experiment.

* $P < 0.001$, † $P < 0.05$, compared to control cultures.

to increase the intracellular concentration of Na⁺. The cardiac glycoside, ouabain, is known to inhibit specifically the Na, K-dependent adenosine triphosphatase [(Na⁺ + K⁺)ATPase], thereby preventing Na⁺ efflux in exchange for K⁺ influx⁹. Our observation that high K⁺ reversed the inhibition by ouabain of PTE-stimulated bone resorption supports the argument that the ouabain effect described here is due to inhibition of (Na⁺ + K⁺)ATPase activity in bone. K⁺ has been shown to compete in a complex manner with ouabain for binding to the (Na⁺ + K⁺)ATPase and to antagonize specifically the effects of cardiac glycosides in other target tissues¹⁰. Also, 0.3 mM ouabain maximally inhibited (Na⁺ + K⁺)ATPase-dependent ⁸⁶Rb uptake by calvaria (N. S. K., unpublished observations).

Monovalent cation ionophores allow fluxes of Na⁺ and other monovalent cations along electrochemical gradients across the plasma membrane of the cell^{4,5}. Ionophore-mediated Na⁺ influx is unrelated to the influx that occurs via the voltage-dependent Na channel. However, veratridine binds directly to the Na channel, stabilizing it in the open conformation and thereby increasing Na⁺ influx¹¹.

Once the intracellular Na⁺ concentration is increased sufficiently to decrease the magnitude of the transmembrane Na⁺ concentration difference, the Na⁺ gradient across the plasma membrane would no longer be favourable for the Ca²⁺ efflux to occur that is coupled to Na influx^{8,12}. If PTH-stimulated bone resorption requires Na–Ca exchange, inhibition of that exchange by increasing intracellular Na⁺ would explain the common effects of the agents we used to inhibit bone resorption. Alternatively, ouabain, veratridine and the ionophores or high

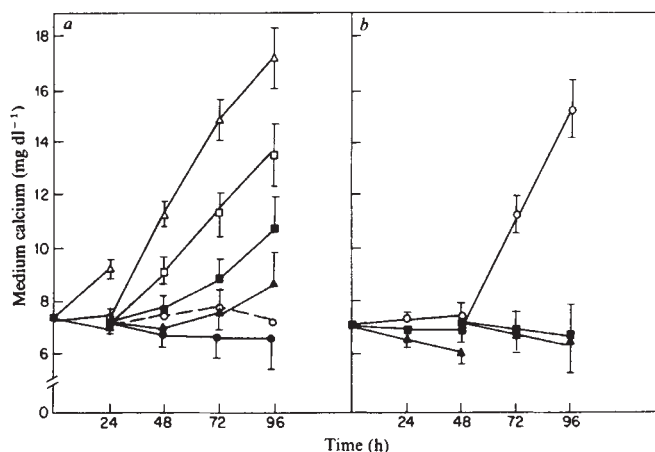


Fig. 1 Effects of pretreatment and reversibility of bone resorption inhibited by nigericin. *a*, Calvaria were cultured as described in Table 1 legend. Bones were treated with 0.1 U ml⁻¹ PTE without or with 10⁻⁷ M nigericin. Medium was changed at 24 h and fresh hormone and ionophore readded after washing each calvarium twice with 2 ml control medium. ○, Control; △, PTH alone; □, control medium for 24 h, then PTH; ■, PTH + nigericin for 24 h, then PTH alone; ▲, nigericin for 24 h, then PTH alone; ●, PTH + nigericin. *b*, Cultures were prepared and incubated in parallel with those in *a*, except that medium was changed and calvaria washed at 48 h. ○, Control for 48 h, then PTH; ■, PTH + nigericin for 48 h, then PTH alone; ▲, nigericin for 48 h, then PTH alone. Values are the mean ± s.e. for at least four bones per group.

intracellular Na⁺ concentrations might disrupt some other critical step in the mechanism of action of PTH by an indirect effect on cell metabolism. However, the findings given in Table 2 make this less likely. The transmembrane Na⁺ concentration difference was decreased by lowering extracellular Na concentration. This manoeuvre had the same effect as increasing intracellular Na⁺, providing strong evidence to support the necessity for a Na–Ca exchange mechanism in the PTH-stimulated release of Ca from bone. A recent model of how cardiac glycoside inhibition of the (Na⁺ + K⁺)ATPase affects Ca²⁺ levels in cardiac muscle cells proposes a similar coupling between a Na–Ca exchange mechanism and the action of the Na pump to regulate transmembrane cation movements⁹.

Table 2 Effects of medium Na⁺ and K⁺ concentrations on PTE-stimulated bone resorption

Medium		Medium calcium (mg dl ⁻¹)			
[Na ⁺]	[K ⁺]	Control	PTE	PTE + ouabain	PTE + nigericin
(mM)	(mM)				
150	5	7.2 ± 0.38	10.4*	6.3*	6.7†
120	5	4.9 ± 0.25	6.4*	5.6†	ND
100	5	4.1 ± 0.25	4.7†	ND	ND
50	5	5.6 ± 0.36	6.0	6.0	6.2
150	0	6.4 ± 0.50	9.5*	4.7	5.4
150	10	5.6 ± 0.48	9.1*	7.5‡	5.5
150	30	7.0 ± 0.28	11.0*	8.4*	5.2*

Calvaria were cultured as described previously⁹ except that the medium was made using an MEM-Select-amine kit (Grand Island Biological Co.) and salt concentrations were adjusted to the desired compositions shown. Isotonicity was maintained using choline chloride whenever the NaCl concentration was decreased below 150 mM. Bones were treated with 0.1 U ml⁻¹ PTE without or with 0.3 mM ouabain or 10⁻⁷ M nigericin and medium was changed once at 24 h. Values given are the mean ± s.e. of four bones per group after 72 h of treatment. As in Table 1, the s.e. for each different medium tested is presented with the control value. The concentrations of Na⁺ and K⁺ in unmodified medium are 150 and 5 mM, respectively. ND, Not determined.

* $P < 0.001$, † $P < 0.05$, ‡ $P < 0.01$, compared to control for each different medium.

The time dependence of the reversibility of nigericin (and ouabain) inhibition, and of the pretreatment effects on PTE-stimulated resorption are also consistent with a mechanism involving an increase in intracellular Na^+ . Coupled transport of Na^+ and amino acids or sugars has been described¹³. Altering the Na^+ gradient would affect this transport system and, with prolonged treatment, such transport would decrease and cellular energy metabolism would be inhibited. This might explain the irreversibility of prolonged inhibition by the agents used. Such a general effect on cellular metabolism is unlikely to explain the inhibition of PTE-stimulated resorption, however, because the inhibition of resorption occurs more rapidly (within 6 h)¹⁴ than the more long-term changes in metabolism.

The effects of pretreatment with nigericin and ouabain imply that their inhibitory actions are not the result of a direct competitive interaction at the level of the PTH receptor. This conclusion is supported by the ability of these compounds to inhibit bone resorption stimulated by prostaglandin E_2 and 1,25-dihydroxyvitamin D_3 as well as PTH¹⁴. Therefore, it seems that $\text{Na}-\text{Ca}$ exchange may be the final common step in the mechanisms of action of several hormones that stimulate bone resorption.

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Disruption of optic fibre growth following eye rotation in *Xenopus laevis* embryos

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Eye rotation is an experimental paradigm used to study axial specification of the amphibian retina and its connections to the tectum¹⁻⁴. Jacobson reported⁴ that the naso-temporal and dorso-ventral axes are determined sequentially and independently between Nieuwkoop-Faber (NF)⁵ stages 28 and 32 in *Xenopus*. Others claim, however, that both retinal axes are determined much earlier and not independently in *Xenopus* and *Rana*⁶⁻⁸. We now find that in *Xenopus* this operation can disturb the exit of optic fibres from the retina with the severity of disruption depending on the time of surgery. Eyes rotated 180° at NF stage 26-27, before any fibres develop, form aberrant retinal fascicle patterns in which the first optic fibres to differentiate (pioneer fibres), failing to exit from the eye, are deflected into circling instead of radial trajectories. Optic fibres appearing later follow these early misguided axons, creating circular bundles. Eyes rotated later (NF 32-34), after optic fibres differentiate, develop normal but inverted patterns because

radial fascicles in the retina at the time of operation accurately guide all newly arising fibres to the optic nerve head. This stage-dependent sensitivity of retinal fascicle development must be considered when interpreting the results of rotation experiments.

While testing Sperry's hypothesis of neuronal specificity in *Xenopus*, Jacobson⁴ claimed that after 180° rotation of the embryonic eye at NF stage 28, the retina regulated, and a normally ordered retinotectal projection developed, whereas rotation at stage 30 led to inversion of only the naso-temporal axis. Rotation at stage 32 produced a completely inverted retinotectal projection with both axes rotated. Others⁷ find in *Xenopus*, however, that rotation of eye vesicles at stage 21-22 gave either rotated retinotectal maps or compound eyes, one normal and the other rotated. Likewise in *Rana*, at Shumway⁹ stage 16-17 and at stage 26 in *Xenopus*, eye rotation resulted in inverted retino-tectal maps^{6,8} with the map's orientation matching the position of the choroid fissure.

In such studies in *Xenopus*, ganglion cell fibres are assumed not to develop until stage 33 (refs 5, 10, 11). Hence, all eye operations before that stage are assumed to be completed before any optic fibres appear in the retina. We found, however, that optic fibres develop early in *Xenopus*¹²⁻¹⁴. Fibres appear as early as NF 28, before the retina completes invagination. They form bundles which enter the optic stalk and reach the brain by NF 32. These bundles organize into a stereotyped radial pattern within the early embryonic retina¹². Evidently, optic fibres differentiate in the retina and form bundles precisely during those stages when the retina is assumed to undergo axial specification⁴. Thus eye rotation before or after the appearance of retinal fibres may have different effects on development of fascicle patterns and may, in turn, affect fibre outgrowth and connections in the brain. For example, the presence of degenerating axons in the optic stalk may guide regenerating axons to their targets in the brain¹⁵.

To determine how the presence of optic fibres at the time of the operation affects development of fascicle patterns, embryonic eyes were rotated 180° at early stages (NF 26-28), before fibres had developed, and at later stages (NF 32-34), after optic fibres had entered the optic stalk and diencephalon. Control operations involved removal of the eye and replacement in normal orientation. When embryos reached tadpole stages 54-55, the operated eyes were removed and the retinas prepared as silver-stained whole mounts to reveal the fibre patterns^{12,16}. A similar whole-mount technique has been used for the developing chick retina¹⁷⁻¹⁹. Silver-stained serial sections²⁰ of embryos fixed at hourly intervals after surgery were also prepared to determine how optic fibres emerge from rotated eyes.

A normal retinal fascicle pattern is shown in Fig. 1. A lesion at the dorsal pole of the eye identifies the dorsal quadrant. The long, thin fascicles in this quadrant are arranged in a distinctive arcuate pattern that differs from the pattern of shorter, thicker bundles in the ventral quadrant. Nasal and temporal quadrant fascicle patterns are very similar.

Twelve of 14 retinas in control eyes operated at early NF stages 26-28 gave rise to normal patterns (Table 1). On the other hand, 13 of 25 early rotated eyes were quite abnormal with bundles of circling axons (Fig. 2). Two retinas were normal in orientation and symmetry whereas eight eyes (one-third) exhibited 90°-rotated or 180°-rotated patterns (Fig. 3). Although some retinas possessed an optic nerve, those retinas exhibiting circling fibre patterns usually had no optic nerves or abnormally thin nerves.

The absence of optic nerves on some retinas indicates that optic fibres did not emerge. In silver-stained serial sections of early rotated eyes we found that a choroid fissure developed dorsally in most instances but no choroid or an abnormal choroid fissure developed in some embryos. Though some fibres exited through dorsally located fissures, others remained within the optic fibre layer or penetrated and grew in the receptor layer (Fig. 4) or over the cornea, sending branches into the dorsal head mesenchyme.

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Table 1 Retinal fibre patterns in 180° rotated eyes

Stage of operation	Series	No. eyes* stained	Normal patterns rotated through			Abnormal patterns		
			0°	90°	180°	Circling fibres	Crossing† fibres	Diffuse‡
Early (NF stages 26–28)	Control	14	12	0	0	2	—	—
	Experimental	25	2	1	7	13	1	1
Late (NF stages 30–33)	Control	12	11	0	0	—	—	1
	Experimental	18	0	4	13	—	—	1

Xenopus embryos reared at room temperature were anaesthetized in 100% Steinberg's solution containing MS 222 (1/3,000). The left eye was cut out of the orbit with tungsten needle and hair loop, then replaced in the orbit without rotation (controls) or rotated 180° (experimentals). Healing for 1 h was assisted by placing a small glass bridge over the eye. Animals were placed in 10% Steinberg's and allowed to develop to late-stage tadpoles (55–58) when they were re-anaesthetized. A lesion was made in the dorsal pole of the retina with a tungsten needle or a Biolaser beam. The animal was killed and the left eye removed from its orbit. After fixing in formol (10%)–sucrose (0.1M) for at least seven days, the lens, cornea, sclera and pigmented epithelium were carefully dissected away. The clean retina was then slit radially in four places and prepared as a whole flat mount. It was stained with silver according to a modified Bielchowsky procedure¹⁶ and mounted in glycerol on a slide. Orientation of each retina was related to the position of the dorsal lesion. Each specimen was photographed in reflected light in a Zeiss Universal photomicroscope with a 4X Zeiss Epiplan HD objective and 6X eye piece. Kodak 4×5 Ektaplan negatives were prepared as contact prints giving a final magnification of almost ×40. To analyse bundle patterns the whole mounts were examined in photographs, under the stereomicroscope and under the light microscope using transmitted light.

* Data based on whole mounts of NF stage 55–58 tadpole retinas.

† A few fibres crossed others.

‡ Patterns stained too diffusely to interpret.

In eyes rotated at NF stages 32–34, 11 of 12 control eyes were normal, but most rotated eyes developed rotated patterns; 95% of fascicle patterns were approximately normal but rotated. Most of these (13/18) were inverted 180° whereas others were rotated only 90°. No normally oriented patterns were obtained although fascicle patterns in each retinal quadrant were normal for that quadrant. All retinas had optic nerves of normal thickness.

The emergence of fibres from eyes rotated at late stages was more normal. In serial sections of embryos examined 4–24 h after rotation, optic fibres emerged through a dorsal fissure. No fibres emerged through ectopic sites or grew into the receptor layer. In only one case did fibres remain in the head mesenchyme and collect into a thick neuroma. Otherwise, fibres leaving such a dorsally oriented fissure penetrated the region occupied by oculomotor nerves and eye muscles.

Thus, rotation of the embryonic eye anlage at early stages before fibres appear, and not the surgical procedure itself, affects development of retinal fascicle patterns. Most patterns are abnormal; the one-third normal but rotated patterns,

resembling those obtained after late rotation, may have been older eyes (stage 28–29) with a few fibres already present at the optic nerve head¹⁴. Staging of early embryos is somewhat unreliable and such staging errors could occur¹³. Also, the two normal patterns may be partly due to errors in the operation or to derotation¹⁹—rotated eyes may return to a normal orientation during healing. Rotation at later stages, after fibres have emerged from the retina and entered the stalk, resulted in normally organized but inverted patterns. Of the combined 43 experimental eyes, only two yielded a normally oriented fascicle pattern.

We suggest that circling fibre patterns develop in eyes rotated early, before fibres are present, because axons that arise after the operation are unable to emerge and consequently grow abnormally within the retina. This hypothesis is consistent with the observation that retinas with the most abnormal circling fibre patterns usually lack an optic nerve and it suggests that fibre outgrowth shortly after rotation is abnormal because the surgical procedure and tissue healing disrupt proper orientation of the first ganglion cell fibres. As the first ganglion cells

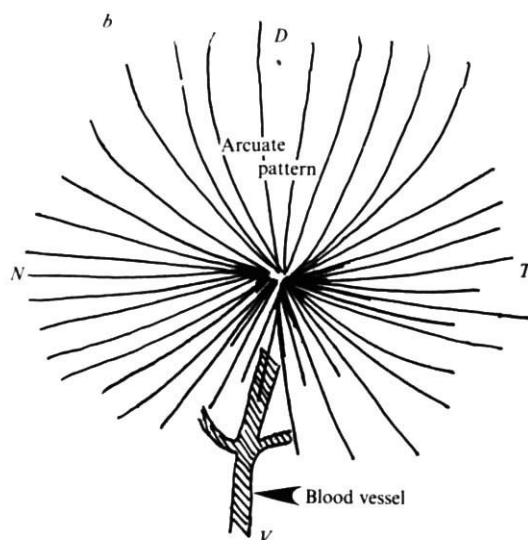
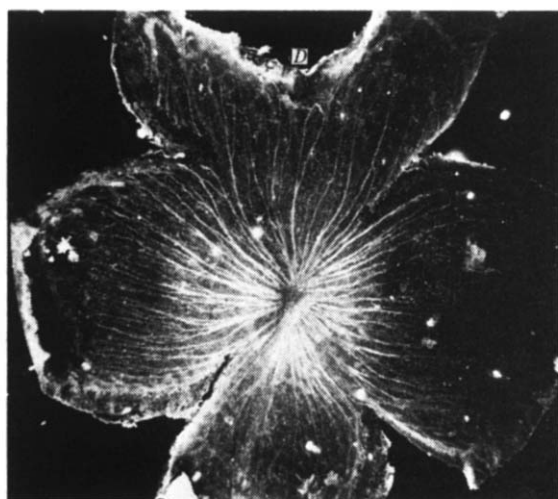


Fig. 1 *a*, Normal fascicle pattern in the optic fibre layer of a silver-stained whole mount of a NF stage 58 *Xenopus* retina. *b*, Diagram to assist identification of specific fascicle patterns. The characteristic thin, long, arcuate fascicles in the dorsal quadrant can be distinguished from the shorter and thicker bundles in the ventral quadrant. The nasal and temporal bundle patterns are mirror images of one another. The ventral blood vessel (hyaloid artery) in the retina is stained lightly and is useful as another orienting marker. Orientation is based on the notch (*D*) located at the dorsal pole of the retina. Magnification ×30. *D*, *V*, *N* and *T* refer to dorsal, ventral, nasal and temporal poles, respectively, corresponding to the position of the eye in the orbit.

differentiate, their axons are guided to the dorsally orientated fissure, if present, or randomly to some other retinal site, if the fissure is abnormal or absent. A few fibres may penetrate the stump of the optic stalk attached to the eye and enter the head mesenchymal space. If closure of the dorsal fissure is abnormal or delayed, or if the stump has healed over before fibres reach the fissure, then many of the earliest fibres do not emerge and grow within the retina instead. As newly differentiating axons at

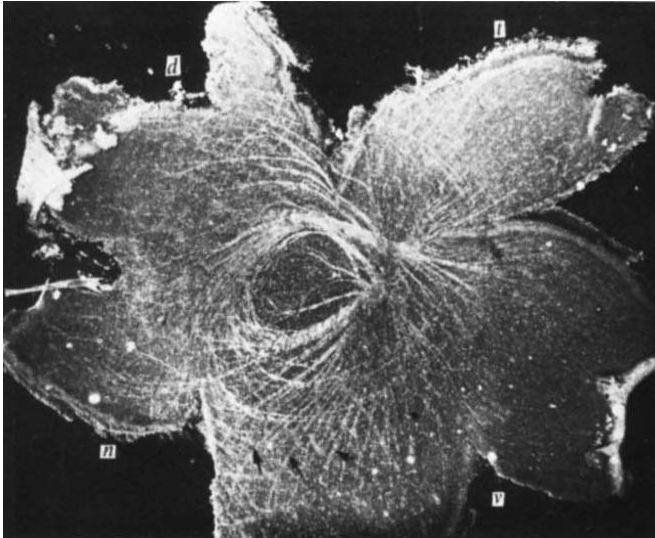


Fig. 2 Early rotation. Eye rotated 180° at stage NF 27/28 and prepared as a silver-stained whole mount at stage 56. Pattern of fascicles is highly abnormal with many fibres circling on themselves within the optic fibre layer of the retina. Some fibres do organize into radial fascicles (arrows) but their number and thickness is very much reduced. *d*, *v*, *n* and *t* refer to dorsal, ventral, nasal and temporal poles, respectively, corresponding to the position of the eye in the orbit. $\times 30$.

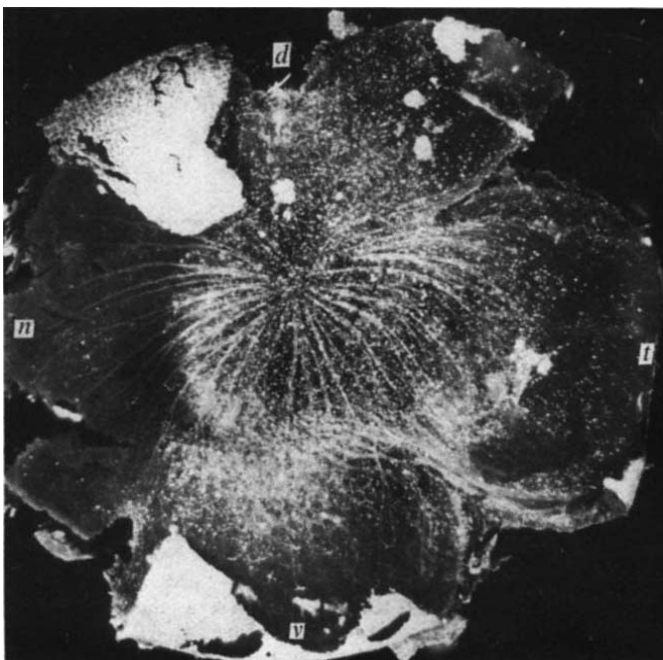


Fig. 3 Late rotation. Eye rotated 180° at NF stage 33 and prepared as a whole mount at stage 56. A normal but 180° inverted fascicle pattern is obtained. Arcuate fascicles characteristic of dorsal quadrant now oriented in opposite direction towards ventral pole. *d*, *v*, *n* and *t* refer to dorsal, ventral, nasal and temporal poles, respectively. $\times 40$.

the margin of the growing retina follow pre-existing fibre bundles¹², only a few abnormally directed pioneering fibres appearing at early stages are sufficient to generate masses of circling fibres. Comparable circling fibre patterns have been noted in the regeneration of sensory fibres within the insect epidermis when regrowth of cuticle prevented fibres from returning to the central nervous system²¹. It remains to determine why the first fibres trapped in eyes form circular patterns, rather than random tangles.

At later stages (NF 32), eyes have formed a normal fissure and fibres have already entered the optic stalk. Fibre pathways to guide ganglion cell axons to the optic nerve head and out of the fissure are thus established before the operation. After eye rotation, fibres in the proximal stump of the optic stalk grow out before wound closure and enter the head mesenchyme. All subsequent ganglion cell axons developing in the inverted eye are guided along the pre-existing fascicles to the optic nerve head, into the proximal stalk and out into the head mesenchyme.

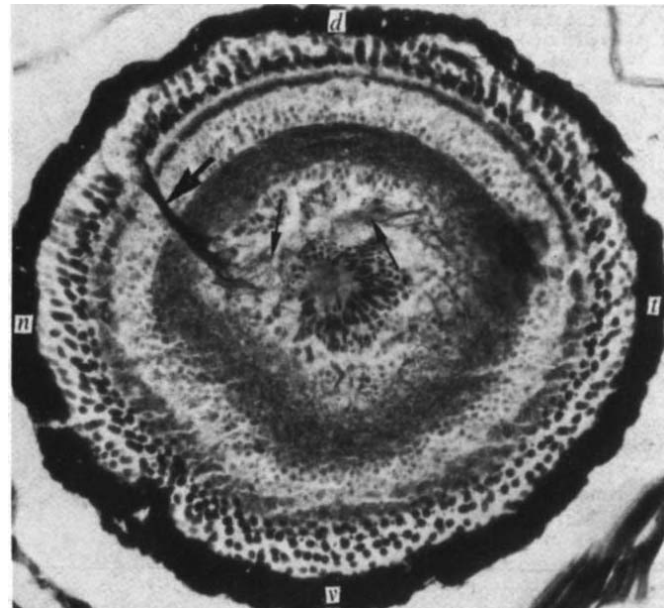


Fig. 4 Early rotation. Left eye rotated at NF stage 26/27 and fixed in aqueous Bouins at NF stage 48. Animal embedded in paraffin, sectioned in the sagittal plane, and stained with silver according to a modified Holmes stain²⁰. Axons inside optic fibre layer of retina (small arrows) circle and collect into a large bundle (large arrow) which penetrates the receptor layer ectopically in dorso-nasal quadrant, at some distance from optic nerve head. Fibre bundle follows receptor layer at periphery when traced into more lateral sections. Some fibres eventually emerge abnormally and enter the dorsal mesenchymal space around the eye. *d*, *v*, *n* and *t* refer to dorsal, ventral, nasal and temporal poles, respectively. $\times 180$.

They reach the brain following aberrant pathways in most cases, usually along the oculomotor nerves or branches of the trigeminal nerve to the hind brain²². As quadrant-specific patterns of retinal growth are unaffected by eye rotation, a normal but inverted fascicle pattern develops within the retina.

These results are consistent with the idea that two processes govern the development of optic fibre fascicles in the retina: fibre following and the kinetics of annular ring growth at the retinal margin¹². These rules are invoked in space-time models ordering growth and connection of retinal ganglion cell axons to visual centres in the brain^{12,23}. It seems that most mapping experiments concerned with retinotectal connections and axial specification need to be re-examined in terms of the effects of surgery on development of retinal fascicle patterns and the emergence of optic fibres from the eye. Experiments correlating

fascicle patterns and optic fibre pathways with retinotectal mapping from experimentally modified retinas are under way.

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Aspartate and glutamate as possible neurotransmitters of cells in layer 6 of the visual cortex

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Earlier work¹⁻⁸ has suggested that aspartate, glutamate and γ -aminobutyric acid (GABA) act as transmitters in the cerebral cortex. There is reasonable evidence for the identity of the cell population responsible for GABA release⁹⁻¹² but until now there has been little evidence concerning the sources for release of aspartate and glutamate. Here we have used two approaches to identify possible neurotransmitters used by cells in the visual cortex: measurement of the efflux of endogenous compounds in conditions of synaptic release and localization of these compounds to particular cell classes using neurotransmitter-specific histochemical techniques. Our results suggest that the acidic amino acids aspartate and glutamate may be cortical neurotransmitters, as shown by calcium-dependent release from endogenous stores and by uptake specific to pyramidal cells in layer 6 of the cortex. These substances may therefore have a role in the function of layer 6 cells, which are responsible for the recurrent projection from the cortex to the lateral geniculate nucleus and for the projection within the cortex from layer 6 to layer 4.

The release experiments were carried out with a tissue-slice preparation from rat visual cortex. An elevated potassium medium was used to induce synaptic release, and a similar medium with low calcium and high magnesium, which should block synaptic release, was used as a control. The release of endogenous compounds from slices incubated in each of these two media was measured using a HPLC system. The system used was capable of detecting compounds containing a primary amino group, with a detection limit of about 10 pmol. Its principal advantage in the present study was to enable us to analyse substances released from endogenous stores in the

tissue. Rates of release for a series of amino acids measured in stimulated and control conditions are given in Table 1. To illustrate more clearly the changes in release between the stimulated and control media, the ratio of the rates of release for the two conditions are shown in Fig. 1. Marked increases were observed for aspartate (12-fold), glutamate (14-fold) and GABA (sixfold). The ratios for the other amino acids ranged from one- to twofold. These findings are consistent with previous work¹⁻⁸.

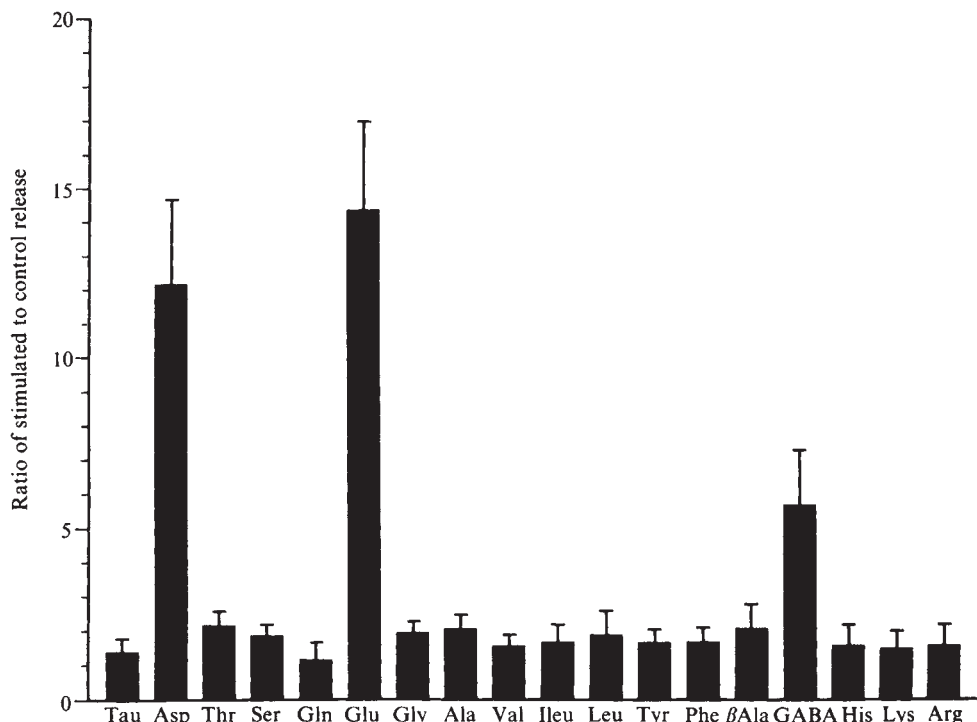
Autoradiographic localization based on uptake of ³H-L-aspartate and ³H-L-glutamate has been relatively unsuccessful for labelling of cell bodies, possibly because these substances are rapidly metabolized following uptake. The D-form of aspartate, which is not metabolized, is accumulated in brain tissue through the same high-affinity uptake system that transports the L-forms of both aspartate and glutamate and thus offers the possibility of labelling aspartate- or glutamate-accumulating neurones¹³. For this reason we used ³H-D-aspartate to label the cortical cells that may be responsible for the previously demonstrated aspartate and glutamate release. Although it should be possible to label these cells at the site of injection, they may also be labelled by neurotransmitter-specific retrograde transport following injection at their distant efferent targets^{14,15}. As cells in different cortical layers have distinct projections, we made injections in

Table 1 Release of various amino acids in stimulated and control conditions

	Release rate (pmol per mg wet wt per 5 min)	
	Stimulated	Control
Taurine	49 ± 16	37 ± 15
Aspartate	206 ± 35	17 ± 4
Threonine	74 ± 14	34 ± 5
Serine	171 ± 54	88 ± 21
Glutamine	76 ± 29	63 ± 9
Glutamate	552 ± 171	38 ± 7
Glycine	98 ± 12	50 ± 10
Alanine	115 ± 22	54 ± 9
Valine	10 ± 2	12 ± 9
isoLeucine	21 ± 6	12 ± 3
Leucine	39 ± 14	21 ± 5
Tyrosine	6 ± 2	4 ± 1
Phenylalanine	10 ± 3	6 ± 1
beta-Alanine	5 ± 2	3 ± 2
GABA	111 ± 25	20 ± 6
Histidine	20 ± 4	14 ± 5
Lysine	19 ± 4	13 ± 6
Arginine	11 ± 6	7 ± 2

Blocks of visual cortex were sliced at 200- μ m thickness with a tissue chopper. The tissue slices were suspended in an Earle's bicarbonate-buffered salt solution (143 mM Na⁺, 5 mM K⁺, 1.8 mM Ca²⁺, 0.8 mM Mg²⁺, 125 mM Cl⁻, 26 mM HCO₃⁻, 1.0 mM H₂PO₄⁻, 0.8 mM SO₄²⁻, 5.5 mM glucose) gassed with 5% CO₂/95% air at 37 °C. The slices were incubated in a series of buffers (5 ml of each) with changes every 5 min. For the stimulated release experiments, the slices were incubated with three consecutive 5-min changes of normal medium followed by a 5-min incubation (stimulated release) in high-potassium (50 mM K⁺, 98 mM Na⁺) medium. For the control release experiments, the slices were incubated with two 5-min changes of normal medium, followed by a 5-min incubation in low-calcium (0.1 mM Ca²⁺, 10 mM Mg²⁺, 132 mM Na⁺) medium and a 5-min incubation (control release) in high-potassium, low-calcium (50 mM K⁺, 0.1 mM Ca²⁺, 10 mM Mg²⁺, 87 mM Na⁺) medium. All buffer solutions contained 1 μ M *n*-leucine as an internal standard. At the end of the incubation series the sections were weighed, and the fractions from each buffer change were frozen in liquid nitrogen, lyophilized and desalted in 6 M HCl in acetone. The compounds present in the final extract were analysed on a HPLC, with a sulphonated divinylbenzene polystyrene cation exchange column (Dionex DC-5A resin in a 4 mm × 15 cm stainless steel column), and *o*-phthalaldehyde fluorescence detection¹⁹. This procedure detects compounds containing primary amino groups, such as amino acids and many peptides. Quantification was based on peak heights compared with the *n*-leucine internal standard.

Fig. 1 Ratios of the rates of release of endogenous amino acids in stimulated and control conditions (from Table 1).



several areas to determine the population(s) capable of transporting the label retrograde from their terminals. These areas included the lateral geniculate nucleus (LGN), which receives input from cortical layer 6, the superior colliculus, which receives input from layer 5, and area 19, which receives input from layers 2 and 3 of area 17 (ref. 16). The most striking result was observed after the injections in the LGN (Fig. 2). Many cell bodies in layer 6 were labelled and a diffuse band of label was present in layer 4. No labelled cortical cells were seen after injections in the superior colliculus, and only a few were seen in area 17 after the injection in area 19. Following injections in area 17 the local pattern of cortical labelling was similar to that seen following retrograde transport from the LGN, that is, there was a diffuse band of label in layer 4 and many labelled cells in the deeper layers, especially layer 6. Thus within the visual cortex the ability to accumulate ^3H -D-aspartate seems to be particularly localized in layer 6 cells.

Following injections in the LGN, the cells in layer 6 are probably labelled by retrograde transport. The band in layer 4, however, could represent labelling either of geniculate terminals of the extensive plexus of collaterals of layer 6 cells which arborize in that layer¹⁷. Two lines of evidence support the latter: (1) at the injection site in the LGN the principal neurones are unlabelled, so it is unlikely that they take up the label and transport it to the cortex. (2) After cortical injections there was only a diffuse patch of label in the LGN at the topographically appropriate site, with no labelling of cell bodies. This is consistent with the idea that the geniculate neurones do not take up the ^3H -D-aspartate, and furthermore demonstrates that the layer 6 cells are capable of transporting the label anterograde to their terminals in the LGN. Thus the band of label in layer 4 seen after the injection in the LGN is probably the result of filling of collaterals of layer 6 cells.

Therefore, several results support the specificity of uptake of ^3H -D-aspartate by the layer 6 cortical cells. From the retrograde transport experiments we found that the layer 5 cells and the cells in the LGN, for example, do not take up and transport the label whereas the layer 6 cells do. Furthermore, after injections of label in the cortex the layer 6 cells are the predominant cell class responsible for uptake in the vicinity of an injection encompassing all layers. Previous studies, which have examined changes in high-affinity uptake of ^3H -D-aspartate and in endogenous content of aspartate and glutamate following cortical

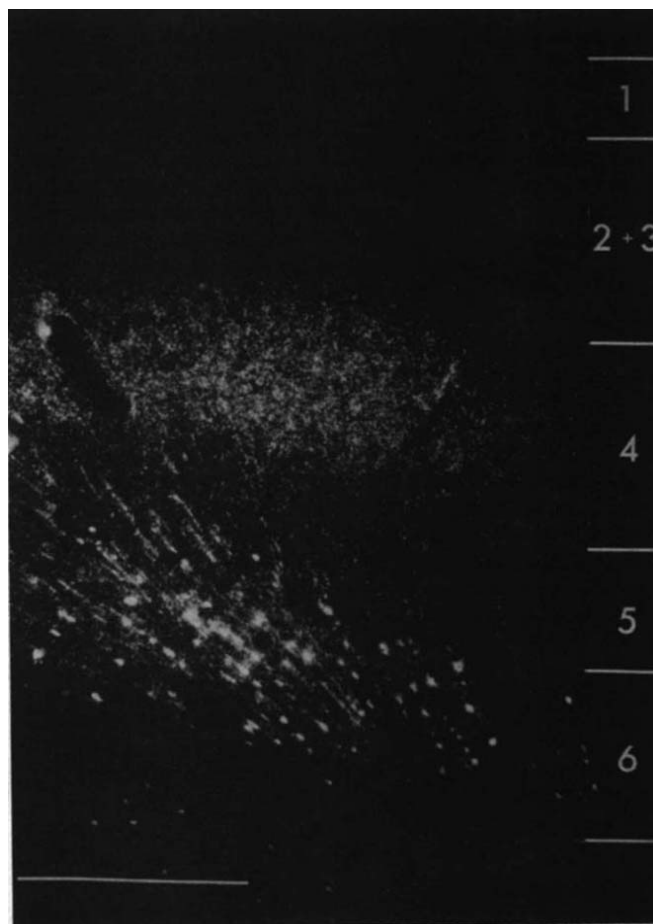


Fig. 2 ^3H -D-aspartate ($50\ \mu\text{Ci}$ in $0.5\ \mu\text{l}$ buffered saline) was pressure-injected from a recording pipette²⁰ into the LGN of the cat. The animal was resuscitated and, after 2 days, re-anaesthetized and perfused with a glutaraldehyde fixative. The brain was sectioned and the sections processed for autoradiography. The figure shows the pattern of labelling in the cortex, with labelled cells in layer 6 and a band of diffuse label in layer 4 (3-week exposure time). Bar, $500\ \mu\text{m}$.

ablation, have suggested that both the corticogeniculate and corticocollicular pathways are associated with aspartate and glutamate¹⁸. The present results suggest, however, that these compounds are only associated with the corticogeniculate pathway.

We have thus provided additional evidence based on release of endogenous compounds that aspartate and glutamate, as well as GABA, may act as neurotransmitters in visual cortex. The above results do not exclude the possibility, however, that aspartate and glutamate may be metabolites of the true transmitter. We have also shown that uptake of ³H-D-aspartate is particularly associated with the cells in layer 6 that participate in the corticogeniculate pathway and that project to layer 4. These findings may be useful in determining the role of this cell class in cortical and geniculate function.

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Cell movements in *Xenopus* eye development

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The vertebrate eye develops morphological markers, such as the ventral choroid fissure, which define its anatomical polarity in relation to the body. Also, retinal nerve fibres behave as though regionally differentiated by forming ordered, topographical maps of the visual field in the brain^{1–4}. It has long been held that these two features of eye polarity—its structure^{5–10} and the neural specificity of the ganglion cells^{11,12}—are labile in the early eye vesicle and become determined relative to body axes at a subsequent stage of development. Contrary to these earlier findings, recent experiments^{13–15} show that the structure and connectivity of the eye are already determined by the time it first appears as a distinct eye vesicle; surgical construction of eye pigmentation chimaeras¹⁴ suggested that normal tectal maps, previously reported after early eye rotations, may have arisen from neural retina cells recruited from the optic stalk after the operation. In agreement, using a radioactive tag which marks neural retina as well as pigment epithelium, I report here that during normal development in *Xenopus laevis*, cells in the ventral retina, where the choroid fissure forms, move into position from the optic stalk region during eye-cup formation. Depending on developmental stage, surgical eye rotations may intercept their movement and this provides a simple explanation of results previously taken to indicate a change from a labile to a determined state of eye polarity.

During development in *Xenopus*, the eye rudiment arises as a lateral protrusion of the neural tube and becomes a distinct eye vesicle at stage 22 (ref. 16). The vesicle, whose epithelium remains connected with the neural tube by the optic stalk until the eye-cup stage, then elongates dorsally leaving the stalk attached to its ventral pole. Before the outgrowth of optic nerve fibres, the vesicle invaginates on itself to form the double-walled eye cup. This inward movement, beginning dorsally at stage 27, brings the presumptive neural retina adjacent to the proximal wall of the vesicle, the presumptive pigment epithelium¹⁷, which attenuates into a thin layer of flattened cells. The choroid fissure develops as a vertical cleft in the ventral part of the eye, beginning at stage 29 in the vesicle margin and elongating to completion along with retinal invagination at stage 32 (ref. 18). Its formation establishes continuity between the inner retinal surface and the optic stalk, thereby providing a direct pathway to the brain for pioneer ganglion cell fibres which begin to leave the eye through the apex of the fissure at stages 30–31 (refs 18, 19). An assumption implicit in the studies using eye rotation surgery to investigate the state of determination of eye polarity in embryos^{5–12} has been that the boundaries of the eye vesicle define those of the newly invaginated eye cup. On this basis, the ventral fissure region should arise from cells already within the eye vesicle. The results presented here show this interpretation of eye morphogenesis, at least in *Xenopus*, to be erroneous.

To trace the cell movements involved in the morphogenesis of the eye cup between stages 22 and 32, a series of experiments was performed in which the whole or part of the *Xenopus* eye rudiment was excised, incubated in a droplet of operating medium containing ³H-thymidine (³H-TdR; 10^{−6} M, 46 Ci mmol^{−1}) for 10–15 min, then washed thoroughly in fresh medium (six changes) and replaced orthotopically in the original embryo. The operating medium was two-thirds strength Niu Twitty²⁰ solution containing 1:10,000 tritacine methane sulphonate (MS 222; Sandoz) at pH 7.6, and the animals were reared at 18–20 °C after the operations. They were fixed 12–48 h later (stages 36–42), serially sectioned at 1–4 μm thickness and prepared for light microscope autoradiography. As the eye, optic stalk and neural tube epithelia are continuous until later eye-cup stages, with no distinct boundaries between them, individual operations unavoidably included a variable number of cells from the optic stalk; however, cuts were made distally as close to the outlines of the vesicle as possible.

Ventral halves of eye vesicles from seven embryos (stages 24–27) were exposed to ³H-TdR in this way. All the autoradiographs showed a labelling pattern similar to that shown in Fig. 1a, which is a stage 36 eye where ventral-half incubation was performed at stage 26. The fissure (arrow), which is prominent in this parasagittal section, divides the ventral retina into two lobes. The ³H-TdR-labelled neural retina cells lie in a band dorsal to the ventral fissure. They do not occupy the ventral retinal lobes, the position expected if the cells within the early eye vesicle give rise to this region. There are sharp boundaries between the heavily labelled incubated cells and the retinal regions above and below. This shows that the sparse grains visible ventrally do not represent the outcome of serial dilution of label by normal cell division in the peripheral retina after the operation, but are due to background and possibly a small amount of diffusion at the time of surgery.

Further evidence for this proximo-distal movement of ventral neuroepithelial cells is presented in Fig. 1b and c. Whole eye vesicles were removed from six embryos during stages 22–28, incubated with ³H-TdR and replaced. All the autoradiographs show label over the entire eye apart from an area of ventral neural retina (Fig. 1b) containing the fissure, except for one at stage 28, where surgery was judged to be deep, leading to label over the whole eye. When, in a 'negative' experiment, the eye vesicle is removed and the whole embryo incubated in ³H-TdR, the autoradiographs show label confined to the ventral retina (Fig. 1c); brain cells are also heavily labelled (not shown). Further preparations of whole eyes labelled after the onset of cup formation at stage 27 gave rise to a progressively smaller

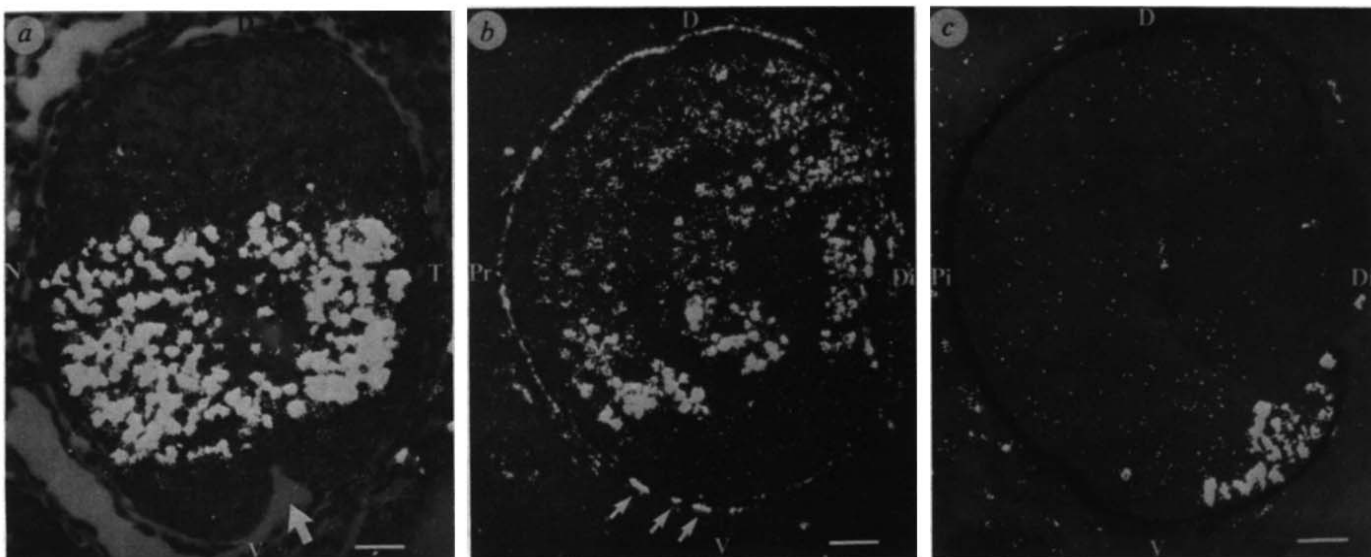


Fig. 1 Autoradiographs of *Xenopus* eyes, using combined light- and dark-field microscopy. Cells containing ^3H -TdR, following *in vitro* incubation in ^3H -TdR (see text), are strongly labelled with dense autoradiographic grains (white) above their nuclei. *a*, Parasagittal section of a stage 37 eye; the ventral fissure is indicated by an arrow. *b*, *c*, Transverse sections of stage 41 eyes from complementary experiments detailed in the text; the arrows in *b* indicate the ^3H -TdR-labelled pigment epithelial cells which are out of register with labelled neural retina (see text). D, Dorsal; V, ventral; ^3H -TdR incubation was performed at stage 26 in *a*, stages 22–23 in *b* and stage 24 in *c*. N, Nasal; T, temporal; Pr, proximal; Di, distal. Scale bars, 30 μm .

area of unlabelled cells occupying the ventral retina, with no such cells appearing after the fissure is fully formed. Therefore, cells positioned in the ventral margin of the eye vesicle do not define the ventral boundary of the newly invaginated eye cup, but are displaced dorsally by the late-arriving cells involved in ventral fissure formation.

Autoradiographs from whole eye incubations frequently show ^3H -TdR-labelled pigment epithelial cells in the otherwise unlabelled ventral region of the eye (Fig. 1*b*). This shows that neural retina and pigment epithelial cells move relative to each other and suggests that the boundaries of the pigment epithelial cell markers used in chimaeric eyes¹⁴ may not reliably define the origin of the underlying neural retina.

As cells which come to comprise the ventral retina are in varying stages of transit from proximal to distal neuroepithelium, 180° eye rotation surgery, thought to reveal the time of polarization of the eye^{5–10}, could give rise to different fissure positions in the eye cup depending on when and where the cuts are made. Deep surgery at vesicle stages, or shallow surgery at early eye-cup stages (Fig. 2*c*) could translocate the entire population of presumptive ventral retina cells dorsally to give a dorsally positioned fissure, whereas shallow surgery at vesicle stages (Fig. 2*a*) might leave them still attached to the stalk, capable, providing healing is successful, of differentiating a normally positioned fissure. Double fissures, dorsally and ventrally located, would result if surgery divided this population of cells in two (Fig. 2*b*). These different patterns of anatomical polarity in the eye have been obtained after rotations at various stages in anuran⁶, urodele^{5,7,8} and chick^{9,10} embryos, and the earliest stage at which dorsal fissures appeared was taken to represent the critical period at which the polarity of the eye becomes determined. As the origin of the fissure-forming cells was not considered in these experiments and was assumed to be in the eye at all stages of rotation, the 'labile' period can simply be explained by incomplete rotation of the whole eye rudiment at vesicle stages.

Jacobson¹² described a critical period during which he thought the polarity of the functional nerve connections from the eye become specified in *Xenopus* (stages 28–31). Although no anatomical evidence was presented, this functional polarization was determined separately from the anatomical polarization of the eye²¹ (but see also ref. 11). However, Gaze *et al.*¹⁴ and

Sharma and Hollyfield¹⁵ systematically failed to isolate a critical stage of polarization and found that even at early stages (stage 22), 180° rotation gave rise to either whole or partly rotated eye anatomy with functional connections of corresponding polarity. Using albino/wild-type chimaeras, Gaze *et al.* showed that eye vesicle rotations frequently result in eyes of compound origin with a rotated dorsal part (graft-derived) and a normally

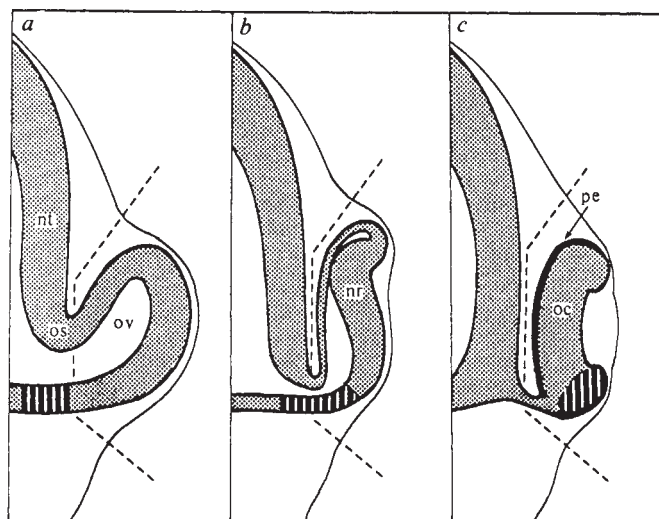


Fig. 2 Schematic representation of eye morphogenesis showing eye rotation surgery (dashed lines) in suggested relation to cell movements in the ventral neuroepithelium (striped region) drawn in transverse section. *a*, Optic vesicle stage (before stage 28 in *Xenopus*). The rotation surgery shown does not include future fissure-forming ventral retina cells. *b*, Invaginating eye at stage of ventral fissure formation (during stages 29–31 in *Xenopus*). The surgery shown divides the ventral cells. *c*, Newly invaginated eye cup (after stage 32 in *Xenopus*) in which the surgery shown includes the ventral retina and fissure. The resulting fissure positions and map ordering are explained in the text. nt, Neural tube; os, optic stalk; ov, optic vesicle; nr, neural retina; oc, optic cup; pe, pigment epithelium.

oriented ventral part (host-derived). They suggested that the unrotated eye tissue, derived from the optic stalk of the host, may have given rise to tectal maps interpreted as evidence for ocular respecification⁹. The present results favour this interpretation, but suggest that the origin of ventral retinal tissue outside the visible outline of the eye vesicle is a normal feature of development, rather than the outcome of cell proliferation stimulated by tissue mismatch caused in eye rotation surgery.

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The cell movements described here may be related to Hirose and Jacobson's observation²², based on cell marking in early cleavage stages of *Xenopus*, that the ventral part of the eye apparently originates from the contralateral neural epithelium.

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Methyltetrahydrofolate is a potent and selective agonist for kainic acid receptors

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Kainic acid, a dicarboxylic acid containing pyrrolidine, is a potent neuronal depolarizing agent in the mammalian central nervous system, being some 50 times more effective than the putative neurotransmitter L-glutamate^{1–5}. As kainic acid is a structural analogue of glutamic acid, it has been proposed that the potent excitatory effects of kainate reflect its restricted conformation, which is optimal for interaction with excitatory glutamate receptors^{2,4}. More recent studies, however, do not support this hypothesis. In invertebrates kainic acid has been found to be a rather weak agonist at excitatory glutamate receptor sites^{6,7}, whereas at the crustacean neuromuscular junction⁸, on certain neurones of *Helix aspersa*⁹ and in the mammalian cerebral cortex³, kainic acid seems to act at receptors distinct from those which mediate the neuroexcitatory action of glutamate. In addition, the ionic requirements for kainate-induced neuronal depolarization differ from those for glutamate-induced depolarization in the mammalian brain¹⁰, and glutamate-induced excitation of rat cortical neurones can be antagonized preferentially by drugs which are very weak in blocking kainate-induced depolarization¹¹. Finally, the characteristics of binding sites for ³H-kainic acid in rat brain are consistent with the existence of specific kainate receptors on neuronal membranes¹². Thus, the kainate sites may represent receptors for an unidentified endogenous substance probably containing the glutamate structure. Here we report that the pteroylmonoglutamate compound methyltetrahydrofolate (MTHF) is a potent competitor for ³H-kainic acid binding sites in rat cerebellar membranes. This action is specific in that MTHF is very weak or inactive at sites for ³H-glutamate or ³H-γ-aminobutyric acid (GABA) respectively. Electrophysiological studies in frog spinal cord suggest that MTHF is an agonist at the kainate receptors. The implications of these results are discussed.

In membranes prepared from rat cerebellum¹³, ³H-kainic acid labelled a single class of receptor sites having a dissociation constant (K_d) of 25 nM. Kainic acid and MTHF displaced 50 nM ³H-kainic acid from these sites with apparent inhibition constants (K_i s) of 0.08 μM and 0.88 μM, respectively (Table 1), and respective Hill coefficients calculated from the displacement data were 0.92 ($r = 0.97$) and 0.94 ($r = 0.99$). L-Glutamate was a relatively weak competitor for the ³H-kainate site ($K_i = 28.3$ μM) and GABA (100 μM) was ineffective in displacing ³H-kainic acid binding.

In the same membrane preparation, ³H-glutamate labelled two populations of receptor. Mean K_d values of 314 nM and 1,850 nM were obtained for the high and low affinity sites, respectively. Binding of 100 nM ³H-glutamate was displaced by glutamate ($K_i = 0.61$ μM) but not by kainic acid (100 μM) or GABA (100 μM). MTHF was a relatively weak competitor for the high affinity glutamate site ($K_i = 83.5$ μM) (Table 1). Binding of 10 nM ³H-GABA to the membranes was displaced by GABA ($K_i = 0.069$ μM) but not by kainic acid, glutamate or MTHF at concentrations of 100 μM.

MTHF is the predominant form of folate in the blood and in cerebrospinal fluid (CSF)^{14,15}. It is taken up into the brain by an active process¹⁶, and subcellular fractionation studies have shown a high concentration in synaptosomal fractions¹⁷. Our data demonstrate that MTHF interacts specifically with kainate receptors on neuronal membranes. In this respect it is almost as potent as kainic acid itself (Table 1). In the frog spinal cord, MTHF induces neuronal depolarization followed by a long period during which the membrane is unresponsive to applied glutamate or electrical stimulation¹⁸. The depolarization induced by MTHF is not antagonized by glutamate diethyl ester, a drug which preferentially blocks glutamate responses¹⁹, and is not effective in blocking kainate-induced excitation¹¹. Thus, MTHF mimics the electrophysiological action of kainic acid in a vertebrate preparation. In addition, MTHF has convulsive properties in experimental animals^{20,21}, and administration of folates to epileptic patients is known to increase the frequency of fits. These observations are consistent with a neuroexcitatory action of MTHF. Thus, MTHF interacts specifically and with high affinity at one class of excitatory receptor sites and has agonist activity at these sites. These are characteristics expected of an endogenous, excitatory, neuromodulatory substance.

Recent studies have shown that *in situ* injection of kainic acid into brain produces a characteristic pattern of neuronal degeneration^{22–25}. The microanatomical selectivity of these lesions together with structure-activity studies on kainate analogues suggest that the neurotoxicity of the compound may be related to its electrophysiological activity^{26–28}. There is some evidence that MTHF is neurotoxic. In vitamin B₁₂ deficiency, there is a marked accumulation of MTHF in the blood and CSF^{29,30}. The occurrence of foci of neuronal destruction in the

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Table 1 Inhibition of kainate, glutamate and GABA receptor binding in rat cerebellar membranes

Agent	³ H-kainic acid binding	K_i ($\times 10^{-6}$ M) ³ H-glutamic acid binding	³ H-GABA binding
Kainic acid	0.08 $\pm$ 0.009	$\gg 100$	$\gg 100$
L-glutamic acid	28.3 $\pm$ 3.60	0.61 $\pm$ 0.07	$\gg 100$
MTHF	0.88 $\pm$ 0.17	83.5 $\pm$ 10.62	$\gg 100$
GABA	$\gg 100$	$\gg 100$	0.069 $\pm$ 0.009
L-proline	—	$\gg 100$	—
Muscimol	—	—	0.020 $\pm$ 0.002

Cerebella from adult Wistar rats were homogenized in 15 vol ice-cold 0.32 M sucrose in a glass homogenizer and centrifuged at 1,000g for 10 min at 4 °C; the pellet was discarded and the supernatant centrifuged at 20,000g for 20 min. This pellet was resuspended in ice-cold distilled water, dispersed with a Brinkmann Polytron PT-10 (setting 6) for 20 s and centrifuged at 8,000g for 20 min. The supernatant fluid was collected and the pellet carefully rinsed to collect the upper layer. This suspension was then centrifuged at 48,000g for 20 min and the pellet washed once in distilled water. Synaptic membranes were stored at -20 °C until use (24–72 h). Frozen pellets were suspended by sonication in 0.05 M Tris-citrate buffer (pH 7.1), centrifuged at 48,000g for 10 min and resuspended in buffer (0.1–0.4 mg protein per ml) for binding assays. Solutions containing MTHF were prepared immediately before use, although the compound is stable for some 12 h at 4 °C. Binding of ³H-kainic acid (2.4 Ci mmol⁻¹, Amersham)¹², ³H-glutamate (21 Ci mmol⁻¹, Amersham)³³ and ³H-GABA (65 Ci mmol⁻¹, Amersham)¹³ was assayed in triplicate centrifuge tubes. Incubation of membranes (0.1–0.4 mg protein) in the presence of ³H-kainic acid, ³H-glutamate or ³H-GABA was continued for 60 min at 4 °C, 15 min at 30 °C, or 5 min at 4 °C, respectively. After incubation, the reaction was terminated by centrifugation at 48,000g for 10 min. The pellets were superficially washed with two 5-ml aliquots of ice-cold buffer and then dissolved by incubating for 30 min at 55 °C in 1.5 ml Protosol (NEN). Dissolved pellets were counted in 10 ml Aquagel I liquid scintillant (Chemlab) in a Packard Tri-Carb 2660 spectrometer with automatic quench correction. Total specific binding of radioligand was defined as the difference between total binding and that in the presence of 0.1 mM unlabelled ligand. IC₅₀ values were determined by log-probit analysis using at least five inhibitor concentrations, and apparent K_i values calculated from the equation $K_i = IC_{50}/(1 + [^3H\text{-ligand}]/K_d)$. K_d values of 25 nM, 314 nM and 22 nM (calculated from Scatchard analysis of saturation isotherms) were used for ³H-kainic acid, ³H-glutamic acid and ³H-GABA binding sites, respectively. Each value is the mean $\pm$ s.e.m. of four experiments each done in triplicate.

brains and spinal cords of B₁₂-deficient patients might be explained on the basis of a neurotoxic effect of excess MTHF. It is interesting that the highest serum MTHF levels are indeed found in patients who have subacute combined degeneration of the spinal cord³¹. In addition, Brennan *et al.*³² have recently demonstrated a selective reduction in high affinity GABA uptake in the brains of B₁₂-deficient fruit bats. This finding suggests a destruction of GABAergic neurones in the B₁₂-deficient state, which is consistent with a neurotoxic action of MTHF.

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Lymphocyte activating factor promotes T-cell growth factor production by cloned murine lymphoma cells

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Evidence has indicated that two soluble factors are essential for a T-cell proliferative response to antigen or lectin^{1–3}. One such factor, thought to be of T-cell origin, is distinguished by its ability to initiate and maintain continuous T-cell growth (T-cell growth factor, TCGF)^{4–6}. A second activity of macrophage origin augments lectin-initiated proliferative responses of thymocytes or adherent cell-depleted splenocytes (lymphocyte activating factor, LAF)⁷. Although it has been suggested that LAF promotes T-cell proliferation by facilitating the production of TCGF by T-cells^{1–3}, direct evidence supporting this hypothesis has been lacking. We describe here experiments with a murine thymic lymphoma which releases TCGF in response to lectin. In serum-free, defined medium, highly purified LAF promotes a concentration-dependent increase in TCGF release. Because the proliferation of T cells depends on the concentration of TCGF available^{8,9}, these results indicate that LAF and TCGF constitute an essential bimodal amplification system which ultimately determines the extent of T-cell clonal expansion.

To approach the functional relationship of LAF and TCGF, we sought LAF-responsive cells which could be cultured in a lymphokine-free medium. WEHI-7, an irradiation-induced Thy 1⁺ lymphoma cell line of BALB/c origin¹⁰ (Salk Institute Cell Distribution Center), was placed into culture with Iscove's modification of Dulbecco's modified Eagle's medium (IMDM) supplemented with bovine serum albumin, transferrin, cholesterol and soybean lipid^{11,12}. The WEHI-7 cells were screened for TCGF production at multiple cell concentrations in the presence of concanavalin A (Con A) or phytohaemagglutinin (PHA) and LAF. No detectable TCGF activity was found in the culture medium in the absence of lectin stimulation, although both PHA- and Con A-elicited TCGF activity were found. LAF obtained from lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cells did not cause detectable TCGF production, although LAF increased lectin-dependent TCGF production three- to fivefold. A typical experiment is shown in Fig. 1, where TCGF activity elicited by Con A in the absence and presence of LAF was assayed by the dose-dependent tritiated thymidine (³H-TdR) incorporation of a TCGF-dependent cytolytic T-lymphocyte line (CTL)⁹.

To investigate whether WEHI-7 was heterogeneous with respect to TCGF production, the cell line was cloned by limiting dilution in microtitre plates (0.3 cells per well, Poisson-calculated probability of co-fertile wells <0.05). Forty-one clones were obtained, and when tested for TCGF, 50% of the clones did not produce detectable TCGF activity regardless of whether lectin or LAF was present. In contrast, all of those clones which released TCGF on lectin stimulation also responded to LAF by at least a twofold increment in TCGF production. Thus, the WEHI-7 cells behaved similarly to normal T-cells: both lectin and LAF were required for optimal TCGF production³.

In previous experiments it was observed that the extent of T-cell proliferation was TCGF-concentration dependent^{8,9}. Therefore, if LAF augmented TCGF production, it seemed likely that LAF availability might be rate limiting in the ultimate T-cell proliferative response, especially if the effect of LAF on TCGF production was also concentration dependent. To examine this possibility, LAF partially purified by molecular gel chromatography and isoelectric focusing was added to TCGF-producing and nonproducing WEHI-7 clones in the presence of an optimal stimulatory concentration of Con A. As shown in Fig. 2, LAF clearly promoted a dose-dependent increase in TCGF release from one clone but not from the other clone.

The identification of a murine T-cell lymphoma that produces TCGF in response to lectin stimulation supports the impression that TCGF is T-cell derived. It was necessary to utilize cloned cells to provide such evidence as it was known that T-cell-enriched, normal cell populations required the presence of adherent cells for optimal TCGF production^{2,3,13}; therefore the small amount of TCGF released by T-cell-enriched splenocytes

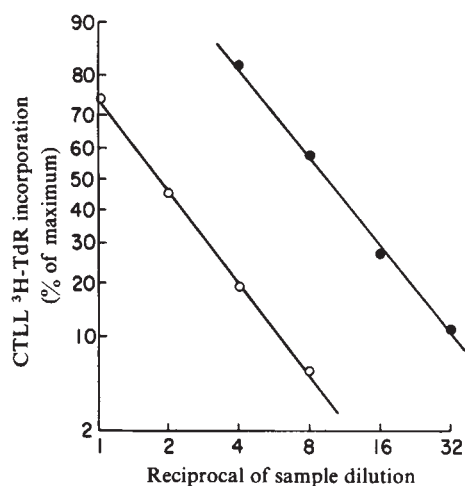


Fig. 1 TCGF activity elicited from WEHI-7 cells by Con A in the absence (○) and presence (●) of LAF. WEHI-7 cells (2×10^6 cells ml^{-1}) were cultured for 24 h in IMDM^{11,12} with Con A ($2.5 \mu\text{g ml}^{-1}$, Miles-Yeda). LAF was obtained from LPS ($20 \mu\text{g ml}^{-1}$, *Escherichia coli*, Gibco-stimulated human peripheral blood mononuclear cells cultured in serum-free RPMI 1640 medium (Gibco) for 48 h. Before use, the LAF-containing conditioned medium was concentrated 60-fold by passage through a YM-10 Amicon filter (Amicon), dialysed against 7,500 vol of saline, 500 vol of RPMI 1640, and assayed for LAF and TCGF activity as previously described^{3,15}. No TCGF activity was detectable in the LAF preparation. The LAF preparation was added to the WEHI-7 cell cultures at a final dilution of 1:1,000. WEHI-7 conditioned medium was assayed for TCGF activity^{3,15} using a TCGF-dependent cytolytic T-lymphocyte line (CTLL-2)¹⁶. The CTLL response to TCGF was quantified and displayed as the reciprocal of the sample dilution which resulted in 50% of the maximum CTLL ³H-TdR incorporation elicited by a standard rat TCGF preparation¹⁵. ³H-TdR incorporation was determined by the addition of $2 \mu\text{Ci ml}^{-1}$ ³H-TdR (specific activity 1.0 Ci mmol^{-1} Schwarz-Mann) during the last 4 h of a 24-h culture period as described previously¹⁵. In the experiment shown, maximum ³H-TdR incorporation yielded 19,500 c.p.m. In the absence of TCGF the CTLL incorporated 75 c.p.m.

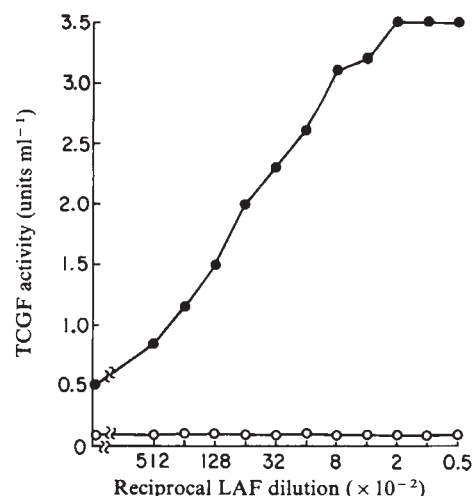


Fig. 2 LAF-concentration-dependent production of TCGF activity from WEHI-7 clone 49 (●) and clone 8 (○). Clones were tested for TCGF production as described in Fig. 1 legend. The LAF preparation was partially purified by Sephadex G-75 chromatography (active fractions migrated with an apparent molecular weight of 20,000) followed by isoelectric focusing over a pH gradient of 3-10. LAF used for the experiment displayed was derived from the isoelectric focusing gradient, pH 6.85. Both the Sephadex G-75 fractions and the isoelectric focusing fractions which contained LAF activity displayed no detectable TCGF activity before addition to the WEHI-7 clones.

could have been derived from residual adherent cells. As cloned thymic lymphoma cells were capable of producing TCGF in the absence of adherent cells and as LAF augments TCGF production, the data indicate that adherent cells contribute an early amplifying signal to T cells capable of TCGF production. Thus, the availability of a clonal population of cells, which can be studied in the absence of serum, provides the cellular system for detailed studies of the mechanism of LAF action and the molecular mechanism of TCGF production. Because both LAF and TCGF are essential for the T-cell proliferative response, such experiments will be important not only to investigate the nature of these lymphokines but also because information gained at the molecular level may increase insight into possible defects of lymphokine function which could underlie disease states.

Because TCGF ultimately mediates T-cell proliferation and because the magnitude of TCGF elaboration is LAF-concentration dependent (Fig. 2), the data indicate that LAF and TCGF function in a bimodal amplification system which finally determines the extent of antigen- or lectin-initiated T-cell clonal expansion. Thus, it may be anticipated that physiological and pharmacological agents known to influence the immune response may do so by altering lymphokine function. Immunosuppressive agents which inhibit LAF-mediated TCGF production would have profound effects on the humoral and cellular immune response, as both cytolytic and helper T-cell proliferation is TCGF-concentration dependent^{9,14}. For example, we have recently found that glucocorticoid hormones inhibit both the production and action of LAF which in turn results in a complete abrogation of TCGF production^{3,5}. Similarly, immunoenhancing agents may function through the LAF-TCGF amplification network. As pointed out by Oppenheim *et al.*⁷, many of the agents used as immunological adjuvants are also potent LAF inducers. As one can study separately the production and action of both LAF and TCGF, the mode of action of immunosuppressive and immunoenhancing agents may be amenable to experimental analysis.

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Alloreactivity of an antigen-specific T-cell clone

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The apparent high frequency of alloreactive T cells has suggested that the two subpopulations—T cells specific for soluble protein antigens and alloreactive T cells—must overlap^{1–3}. However, the existence of a set of germ-line genes encoding a family of anti-MHC (major histocompatibility complex) antigen-specific T-cell receptors has also been postulated to explain the high frequency of alloreactive clones^{4–7}. In these latter models the two T-cell subpopulations are viewed as separate. Studies on whole T-cell populations have examined these two points of view by looking for cross-stimulation of alloreactive cells with antigen and vice versa^{8–11}. Although significant cross-reactions have been reported, the problem of nonspecific recruitment made interpretation of results difficult. Recent developments in the cloning of antigen-specific T lymphocytes^{12–18} have finally allowed investigation at the single-cell level where the results should be unambiguous. von Boehmer *et al.*¹⁴ have isolated a cytotoxic clone specific for the H-Y antigen in conjunction with the syngeneic MHC antigens coded for by D^b. This clone was also cytotoxic for spleen cells bearing the alloantigens coded for by D^d in the absence of the H-Y antigen. However, because cytotoxic clones only grow in the presence of T-cell growth factor (TCGF) and because von Boehmer *et al.* were unable to stimulate or enrich for D^d-specific cytotoxic activity from bulk cultures initially stimulated with H-Y and D^b-bearing spleen cells¹⁴, they hesitate to conclude that the same cell can respond to both foreign and alloantigens. We show here that a (dinitrophenyl-ovalbumin)(DNP-OVA)-specific T-cell clone from a B10.A mouse can be stimulated to divide (and propagate) *in vitro* with B10.S allogeneic spleen cells in the absence of DNP-OVA and B10.A antigen-presenting cells. Extensive recloning strongly suggests that a single T-cell clone is responding to two separate stimuli, a specific alloantigen and a soluble antigen in conjunction with self-MHC products.

B10.A mice were immunized with DNP-OVA and T-lymphocyte colonies prepared from the draining lymph nodes as previously described¹². Most of the colonies tested were shown to be antigen specific, that is, their cells could be stimulated to divide with DNP-OVA but not other antigens such as GAT or purified protein derivative (PPD). One of these colonies was chosen to study alloreactivity as shown in Table 1. Cells from this colony could be stimulated to divide in the absence of DNP-OVA with either B10.S or B10.D2 irradiated spleen cells but not spleen cells from mice of six other independent halo-

types. No change in the results was seen if syngeneic B10.A spleen cells were also added. This latter condition is essential for antigen stimulation although not sufficient by itself (Table 1). These results suggested that an antigen-specific cell could also manifest alloreactivity.

However, this conclusion depends on the colony being a true clone. Although all the colonies grew up completely isolated in the agar and were easy to pick independently of one another, whether each colony derived from a single cell is not clear as a large number of cells (2×10^6) were seeded in the agar allowing for the possibility of recruitment of nearby cells into the colony.

Table 1 Maintenance of alloreactivity on recloning

Source of spleen cells	Proliferative response (c.p.m. $\pm$ s.e.m.)		
	Colony	Clone 5	Subclone 5.6
B10.A	25 (± 5)	150 (± 95)	180 (± 52)
B10.A + DNP-OVA	9,600 (± 680)	14,700 ($\pm 2,300$)	39,200 ($\pm 1,950$)
B10	30 (± 5)	130 (± 55)	233 (± 116)
B10.D2	9,100 (± 600)	236 (± 82)	410 (± 35)
B10.M	10 (± 2)	160 (± 105)	46 (± 8)
B10.P	10 (± 1)	153 (± 42)	230 (± 41)
B10.Q	45 (± 5)	190 (± 150)	60 (± 20)
B10.RIII	25 (± 15)	107 (± 18)	53 (± 12)
B10.S	18,200 ($\pm 2,400$)	15,900 ($\pm 2,000$)	36,300 ($\pm 1,900$)
B10.PL	15 (± 5)	50 (± 25)	80 (± 39)

A colony of DNP-OVA-reactive proliferating T lymphocytes was derived from primed B10.A lymph node cells as previously described¹². Small numbers of cells from this colony were cloned in soft agar with 80% plating efficiency. Of the 14 subcolonies which were picked and expanded, cells from one, clone 5, were again cloned in soft agar, this time with a 91% plating efficiency. Six of these colonies were picked and expanded. The table shows the antigen specificity and alloreactivity of the original colony, clone 5, and subclone 5.6. 10^4 T cells from the original colony and clone 5 or 2×10^4 T cells from subclone 5.6 were mixed with 10^4 irradiated (2,000 rad) spleen cells of various MHC haplotypes in the presence of 5% Con A supernatant. In the test for antigen stimulation, 10% Con A supernatant was used and $100 \mu\text{g ml}^{-1}$ of DNP-OVA was added. The cells were cultured for 6 days in RPMI 1640 plus 10% FCS with supplemental feeding at day 3 (ref. 12). At 16–18 h before collecting, the cultures were pulsed with $1 \mu\text{Ci}$ of tritiated thymidine. The data shown are mean (c.p.m.) $\pm$ s.e.m. for triplicate determinations.

To investigate this problem, 200 cells from one colony were supplemented with 10^4 irradiated B10.A spleen cells and recloned in soft agar¹². The use of a few T cells assured us that all colonies which developed would not have other T cells near them in the agar. Thus we could be sure that each of the colonies was derived from a single cell. After five days of incubation 160 colonies were observed in the agar for a plating efficiency of 80%. Of 15 of these colonies picked and expanded in liquid culture, 14 survived to test for antigen specificity; the remaining colony was lost because of microbial contamination. Cells from all 14 subcolonies showed an identical antigen specificity, responding to stimulation with DNP-OVA but not GAT or PPD (data not shown). Furthermore, as shown for a typical example in Table 1, cells from all 14 subcolonies responded to allostimulation with irradiated B10.S spleen cells. The dose-response curves shown in Fig. 1 indicate that the irradiated B10.S spleen cells could stimulate the B10.A T cells in the

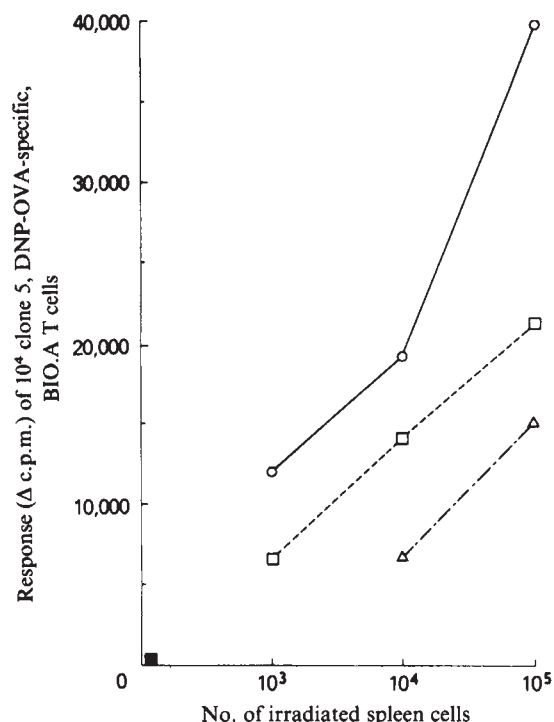


Fig. 1 10^4 T cells from B10.A clone 5 specific for DNP-OVA in association with B10.A spleen cells were stimulated with various numbers of irradiated allogeneic B10.S spleen cells in the absence of Con A supernatant (0% IL-2, Δ) or the presence of 5% (v/v) IL-2 ($\square$) or 10% IL-2 ($\circ$). ■ indicates that no stimulation was seen with 10% IL-2 in the presence of 10^5 irradiated syngeneic B10.A spleen cells.

absence of any added growth factor in the culture (0% IL-2), provided that the T cells were cultured at a high enough density (10^4 per well) and a sufficient number of stimulator cells was added ($\geq 10^4$). However, addition of 5% or 10% concanavalin A (Con A) supernatant to the cultures improved the magnitude of the allogeneic proliferative response at all spleen cell doses, even though these concentrations of Con A supernatant were totally nonstimulatory in the presence of similar numbers of syngeneic B10.A spleen cells. The allostimulation of B10.S spleen cells was not due to a 'back-reaction', that is, recruitment into proliferation of the cloned T cells by alloreactive T cells in the irradiated spleen cell population, because cells from a B10.S macrophage colony were also capable of eliciting a response (data not shown).

Interestingly, none of the subcolonies responded to irradiated B10.D2 spleen cells. This suggests that the original colony was not a true clone but was contaminated with an alloreactive cell specific for H-2^d MHC antigens. The loss of reactivity against H-2^d antigens on recloning made us decide to reclone again to assure ourselves that the remaining alloreactivity for B10.S antigens was indeed associated with the same cells showing specificity for DNP-OVA. In this experiment the cells were recloned in the presence of 25% Con A supernatant in the bottom agar layer to improve the plating efficiency. The number of colonies observed ranged over 170–194 per 200 cells plated with a mean plating efficiency of $91 \pm 2\%$. Six of these colonies were picked and expanded in liquid culture. All six could be stimulated with either DNP-OVA in the presence of syngeneic spleen cells or irradiated B10.S allogeneic spleen cells (Table 1, subclone 5.6). The failure to separate these two activities on recloning suggests strongly that the subcolonies were already true clones and that a single T cell possesses receptors for both specificities.

Previous studies with this B10.A T-cell clone had demonstrated that recognition of DNP-OVA was MHC restricted,

requiring syngeneic B10.A irradiated spleen cells to present the antigen¹⁵. The gene(s) controlling this histocompatibility restriction was mapped to the I-A^k subregion of the MHC. Table 2 shows a similar type of genetic mapping experiment for alloreactivity. Data are shown for one clone but all 14 gave the same result—mapping of the gene(s) to the I-A^s or I-B^s subregion. Because the major lymphocyte-activating determinant (LAD) locus has been mapped to the I-A subregion¹⁹, it is reasonable to conclude that the stimulating alloantigens in this case map to I-A^s. Thus, the same T cell which can respond to DNP-OVA in conjunction with I-A^k is also stimulated by I-A^s. The studies so far do not allow us to determine whether the same receptor or separate receptors account for the two specificities possessed by the clone.

These results have important implications for theories of T-cell recognition, as some of these theories^{4,20} have postulated that alloreactive and antigen-specific T cells are separate subpopulations. Such models could account for the results presented here by postulating that we have identified a rare clone in which the receptor for the antigen, DNP-OVA, happens to cross-react with a determinant on the alloantigen, I-A^s. This is a testable hypothesis as it postulates that in this rare instance, the alloantigen is actually presented to the T cell like a foreign antigen, that is, in conjunction with I-A^k on the syngeneic antigen-presenting cell. As already mentioned, B10.S spleen cells will stimulate the clone in the absence of the additional B10.A spleen cells which are required for DNP-OVA presentation. However, it could be argued that sufficient B10.A spleen cells are present in the T-cell population, as a result of the feeding requirements for propagating the clone, to allow for the presentation of I-A^s antigens.

To test the possibility that I-A^s was recognized in conjunction with I-A^k, we grew one of the clones by stimulating it only with irradiated B10.S spleen cells in the presence of 5% Con A supernatant. At no time during the 39-day propagation were the cells exposed to DNP-OVA or B10.A spleen cells, even when the cultures were split. At the end of this time the cells were retested for specificity. As shown in Table 3, the cells retained their antigen specificity, responding to DNP-OVA and the carrier OVA but not to GAT or the hapten on another carrier, DNP-GLA. They also retained their allospecificity for B10.S and did not acquire any other alloreactivity. To reduce further the possibility that residual B10.A spleen cells remained in the T-cell cultures, the clone was propagated for another 29 days, this time in the complete absence of all spleen cells and antigen. This was done by increasing the concentration of the Con A supernatants (TCGF) to 50% (v/v). Again the clone maintained both its alloreactivity and antigen specificity (Table 3). Thus, after 68 days without adding B10.A spleen cells, presumably a time vastly longer than the lifetime of any irradiated spleen cells,

Table 2 Genetic mapping of allostimulation

Spleen cells		Clone 5 (c.p.m. $\pm$ s.e.m.)
Strain	MHC haplotype	
B10.A + DNP-OVA	kkkkkddd	14,700 ($\pm$ 2,300)
B10.A	kkkkkddd	150 ($\pm$ 95)
B10.S	ssssssss	15,900 ($\pm$ 2,000)
B10.S (9R)	ss?kkddd	15,700 ($\pm$ 800)
A.TL	skkkkkkk	90 ($\pm$ 15)
A.TH	sssssssd	15,700 ($\pm$ 2,300)

10^4 B10.A anti-DNP-OVA clone 5 T cells were stimulated with 10^4 irradiated (2,000 rad) spleen cells from various strains in the presence of 5% Con A supernatants. A 6-day tritiated thymidine incorporation assay was performed as described in Table 1 legend. The data are expressed as c.p.m. $\pm$ s.e.m. for triplicate determinations. The letters in the MHC haplotype column refer to the haplotype source of origin of the K, I-A, I-B, I-J, I-E, I-C, S, G and D regions of the murine MHC. The value for A.TH spleen cells was obtained in a separate experiment carried out in exactly the same conditions.

Table 3 Specificity of an alloreactive clone grown in the absence of antigen and syngeneic spleen cells

Antigen	Spleen cells	Proliferative response (c.p.m. $\pm$ s.e.m.) of clone 5 expanded with:		
		DNP-OVA and B10.A spleen	Allogeneic B10.S spleen	50% Con A supernatant
None	B10.A	90 (± 29)	63 (± 3)	120 (± 6)
DNP-OVA	B10.A	15,700 ($\pm 1,000$)	14,100 ($\pm 2,000$)	14,000 ($\pm 1,200$)
OVA	B10.A	13,700 ($\pm 1,200$)	14,900 ($\pm 1,300$)	14,300 ($\pm 1,170$)
DNP-GLA	B10.A	143 (± 70)	70 (± 17)	50 (± 12)
GAT	B10.A	150 (± 55)	73 (± 26)	50 (± 21)
None	B10.S	15,900 ($\pm 2,015$)	13,800 (± 800)	13,100 (± 400)
None	B10.D2	236 (± 80)	60 (± 15)	90 (± 12)

DNP-OVA-specific B10.A T-cell clone 5, which is normally propagated by feeding every three days with 10^4 irradiated B10.A spleen cells and $100 \mu\text{g ml}^{-1}$ of DNP-OVA in medium containing 20% (v/v) Con A supernatant, was propagated with 10^4 irradiated B10.S spleen cells and 5% Con A supernatant for 39 days followed by propagation with 50% Con A supernatant alone for an additional 29 days. The table shows the antigen specificity and alloreactivity of the original clone (column 1), the clone after being grown with allogeneic spleen cells (column 2) and the clone after being grown with high concentrations of Con A supernatant (column 3). Antigen specificity was assessed in a 6-day thymidine incorporation assay using 10^4 T cells, 10^4 syngeneic, irradiated (2,000 rad) B10.A spleen cells, 10% Con A supernatant and $100 \mu\text{g ml}^{-1}$ of DNP-OVA, GAT and DNP-GLA or $300 \mu\text{g ml}^{-1}$ of OVA (see Table 1 legend). Alloreactivity was assessed by adding 10^5 irradiated B10.S or B10.D2 spleen cells in the presence of 5% Con A supernatant. The data are expressed as mean c.p.m. $\pm$ s.e.m. for triplicate determinations.

the alloreactivity of the clone was not diminished. These results strongly indicate that the T-cell clone is responding directly to I-A* antigens and not to these antigens in conjunction with syngeneic I-A* gene products.

Overall our data demonstrate that individual T cells do exist which can recognize and respond to both alloantigens and soluble protein antigens. This dual specificity can be explained in a number of ways. One could postulate two completely independent sets of receptors randomly expressed in all lymphocytes², alloantigen recognition through high affinity cross-reactions with the anti-self MHC receptor³ or the antigen-specific receptor^{6,7}, or recognition through a combination of both the anti-self MHC and antigen-specific receptors^{8,10}. Further studies are required with more T-cell clones to distinguish between these various models for T-cell recognition.

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A G_1 rate model accounts for cell-cycle kinetics attributed to 'transition probability'

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Smith and his colleagues¹⁻⁶ have proposed that the duration of the cell cycle is determined by a random transition, analogous to the random decay of a radioactive nucleus, by which a cell passes from an 'A state' within the G_1 phase to a 'B phase' that includes the rest of the cycle. The experimental support for this transition probability hypothesis is the tendency of a cumulative plot of differences of cycle times of sibling cells (the β curve) to be exponential and parallel to the exponential tail of a cumulative plot of the cycle times themselves (the α curve). However, a close examination of four of the most extensive sets of experimental data now suggests that the two β curves with the steepest slopes may not, in fact, be exponential. These and all the other characteristics of the experimental curves are best matched by computer simulations using a cell-cycle model that will be termed here a G_1 rate model. This model is consistent with differences in cell metabolism, rather than a transition at an inherently unpredictable time, being the physiological basis for differences in cycle times within a cell population.

As cycle control occurs mainly in the G_1 phase, the formulation of any realistic model requires that the G_1 phase be treated separately from the remainder of the cycle. Monte Carlo simulations of α and β curves were performed using different combinations of exponential, reciprocal-normal, log-normal and normal distributions in two successive phases. Matches to the published curves were most readily obtained with normally distributed reciprocals of G_1 times (called G_1 rates by Siskin and Morasca⁷) and normally distributed times of passage through a second ($S+G_2+M$) phase. If t is the complete cycle time for a cell passing through the G_1 phase at a rate r_1 and through the remainder of the cycle in time t_2 ,

$$t = 1/r_1 + t_2$$

Let ω' and ω'' be random numbers from a normal distribution having zero mean and unit standard deviation, with ω' maintained the same in two sibling cells and ω'' generated separately for each of the siblings. The complete G_1 rate for a single cycle can be written as

$$r_1 = \bar{r}_1 + k_1[c_1\omega' + (1-c_1)\omega'']$$

in which $\bar{r}_1$ is the mean G_1 rate over the entire cell population and c_1 is a fraction determining the degree of sibling correlation. The value of k_1 is related to the standard deviation, σ_1 , of the G_1 rate

$$k_1 = \sigma_1[c_1^2 + (1-c_1)^2]^{-1/2}$$

Similarly, using comparable notation and new random numbers, the complete time of the second phase is

$$t_2 = \bar{t}_2 + k_2[c_2\omega' + (1-c_2)\omega'']$$

$$k_2 = \sigma_2[c_2^2 + (1-c_2)^2]^{-1/2}$$

In Fig. 1, experimental α and β curves (individual points) are matched with computer simulations for 100,000 sibling pairs of cycles using the G_1 rate model (solid and dashed lines). The experimental data are from publications of the Smith group, except for set *b* (ref. 8), which has not been previously plotted as α and β curves. In the four sets of points a downward bend at the top of the β curve⁹ is most pronounced for the steepest curve (*a*) and progressively less pronounced in the three other sets, left to right. The computer simulations for the G_1 rate model show the

Table 1 Ability of G_1 rate and transition probability models to generate β curvatures found experimentally

Data	Pairs	β at midpoint	Experimental		G_1 rate model			Transition probability model		
			Slope	Curvature	Mean slope	Mean curvature	P^*	Mean slope	Mean curvature	P^*
<i>a</i>	37	0.70	-1.328	-0.691	-1.258	-0.476	0.22	-1.276	+0.002	0.02
<i>b</i>	256	0.25	-0.701	-0.288	-0.708	-0.368	0.67	-0.699	+0.005	0.06
<i>c</i>	107	0.35	-0.367	+0.147	-0.371	+0.108	0.58	-0.410	-0.099	0.85
<i>d</i>	24	0.40	-0.156	+0.242	-0.174	-0.069	0.74	-0.187	-0.145	0.78

For each model and each data set, 10,000 β curves were simulated using the same number of sibling pairs as was used experimentally (column 2). Parameters for the G_1 rate model were those of Fig. 1. For the transition probability model, β curves were generated for i cycles from the relationship $t_{ik} = (\ln N_i)/K$, with t_{ik} being the time spent in the A-state, K the measured slope of the experimental β curve, and N_i one of a set of random numbers, distributed uniformly between zero and unity. Each experimental and simulated curve was divided into upper and lower portions at a midpoint (column 3) chosen to delineate effectively the experimental curvature. Slopes (natural log units h^{-1}) were calculated by linear regression, using approximately 1,000 values at equal time intervals along line segments between the data points. Curvature was defined as the difference of the slopes of the upper and lower portions of a curve divided by its overall slope.

* Proportion of the 10,000 simulated β curves having a curvature more negative than the experimental curve.

same progression. For the simulations the major influence on the slopes of the α and β curves is the mean G_1 rate. Varying this parameter over a factor of 4.6 (with some adjustment of other parameters to give optimal matches to experiment) changes the slope of the β curve by a factor of 8. Other combinations of mean and standard deviation can also match the α and β curves. The correct combination could be determined if the mean G_1 time were known, but this was not measured for the experimental cell populations.

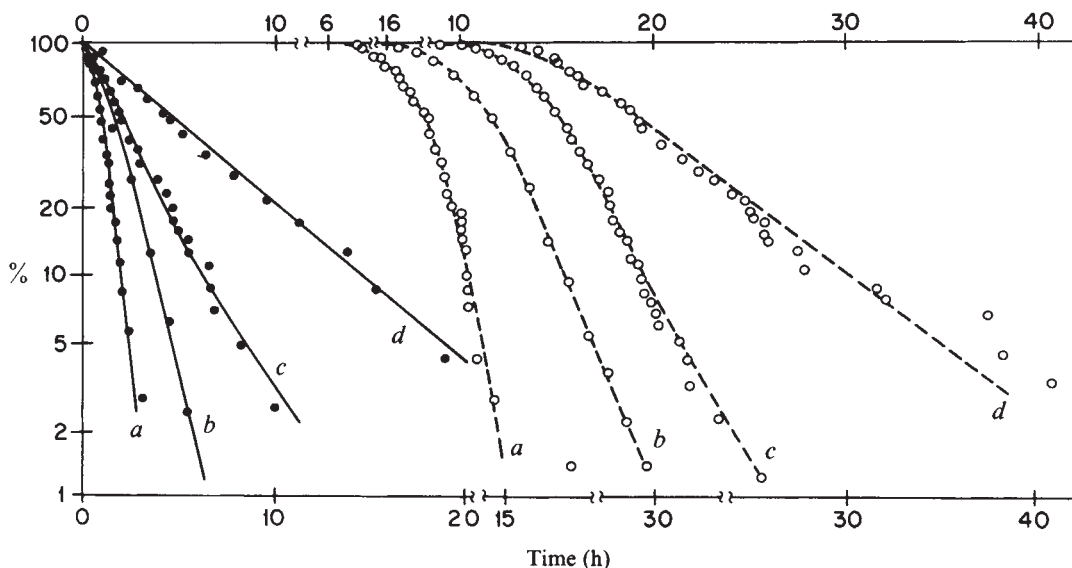
The two sibling correlation factors, c_1 and c_2 , establish the slope of the β curve in relation to the slope of the α curve. The closeness with which a β curve approximates a straight line is enhanced when $c_1 \ll c_2$. This principle corresponds, to a rough approximation, with the assumption of no sibling correlation in the first phase of the transition probability model and perfect sibling correlation in the second phase²⁻⁴. However, the β curvature in set *b* was better matched with c_1 slightly greater than c_2 , suggesting that there is no general rule that the G_1 phases of siblings are weakly correlated.

To examine the role of sampling error in determining β curvature, 10,000 simulations of each β curve were generated for each of the two models, using the same number of sibling pairs as was used experimentally. The proportion of the 10,000 simulated curves having a curvature more negative than the curvature found experimentally was then a measure of the

frequency with which this curvature will be generated by the model. The repeated simulations (Table 1) show that the curvatures of all four sets of experimental data are within the range of values frequently attained with the G_1 rate model, as are the curvatures of sets *c* and *d* for the transition probability model. However, the transition probability model infrequently attains the curvatures of either set *a* ($P = 0.02$) or set *b* ($P = 0.06$). These data do not completely exclude the transition probability model as an explanation of cell-cycle control, but they suggest that a model that gives a slight downward curvature to steep β curves provides a more likely explanation.

These results with a model having a reciprocal-normal distribution in the G_1 phase are in contrast to those of Shields⁴, who constructed α and β curves from 100 simulated cell cycles based on reciprocal-normal and log-normal models and concluded that neither model generated an exponential β curve. Aside from the problem of sampling error in such a small number of simulations, it is not reasonable to compare the two-phase transition probability model with a single-phase model, particularly as the major site of cycle control is known to be in the G_1 phase. By restricting the reciprocal-normal distribution to the G_1 phase, the G_1 rate model achieves higher coefficients of variation and brings the β curvature into a range that can accurately match the experimental β curves. Shields also concluded that only the transition probability model leads to

Fig. 1 Comparisons of published experimental data (●, ○) and Monte Carlo simulations using the G_1 rate model (—, ---) of the % of sibling pairs with a difference of cycle times $\langle t \rangle$ (β curves: ●, —) and the % of cell-cycle times $\langle t \rangle$ (α curves: ○, ---) against time, t . The simulations used 100,000 sibling pairs. They took into account the finite time a culture could be filmed (24, 125, 36, 60 h for *a-d*, respectively). Times between film frames were considered to be negligibly small except for *b*, which was 1 h. (These refinements had little effect on the simulated curves except for depression of both curves



of *d*). Numbers in parentheses are the six parameters ($\bar{t}_1, \sigma_1, c_1, \bar{t}_2, \sigma_2, c_2$) determining the simulated curves. *a*, Ref. 2, Fig. 2 (0.3, 0.03, 0.30, 7.3, 2.0, 0.70); *b*, ref. 8, Fig. 5 (0.131, 0.03, 0.68, 13.3, 2.0, 0.55); *c*, ref. 3, Fig. 1 (0.250, 0.11, 0.0, 10.5, 2.3, 0.70); *d*, ref. 3, Fig. 4 (0.065, 0.03, 0.40, 4.2, 1.0, 0.70). Measured correlation coefficients of simulated cycle times of siblings were: *a*, 0.83; *b*, 0.70; *c*, 0.36; *d*, 0.22.

parallel and exponential β curves when the data are divided into groups according to the shorter cycle time of each sibling pair. However, simulated β curves using the G_1 rate model have the same characteristic, within the sampling error of the experimental data (unpublished results).

Recent experiments using flow cytometry¹⁰ have shown a 10% coefficient of variation in the RNA content of cells in early G_1 phase, comparable with the 10% coefficient of variation in the G_1 rate of set *a*, Fig. 1. The RNA content of individual cells was found to be correlated with the rate of progression through both the G_1 and S phases. Thus, much of the variation of cycle times within a population can be attributed to constitutive variations in the capacity of cells to synthesize protein. Theoretically, there could be a random transition in addition to the variation due to constitutive differences⁵. However, the present results show that the α and β curves can be accounted for completely without postulating such a transition, and there is no independent experimental evidence for one. The most reasonable hypothesis for interpreting the distribution of cycle times in a cell population is that it results from cell-to-cell variations in rates of metabolic reactions and in times required to complete essential steps in the cycle.

I thank Dr David Rigney for devising the original computer program and for many fruitful discussions. The simulations were performed on a PDP-11/70 computer (Digital Equipment Corp.), and the work was supported by DHEW grants CA-06927, CA-22780 and CA-24961 awarded by the National Cancer Institute and by an appropriation from the Commonwealth of Pennsylvania.

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Cytosolic calcium ion activity in epithelial cells of *Necturus* kidney

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Calcium ions are believed to play an important role in the control of cellular function¹, and have recently been implicated in the regulation of sodium and water transport in epithelia^{2–8}. In epithelial cells, as in excitable cells¹, low cytosolic free calcium levels may be maintained, in part, by a process of Na–Ca exchange across the plasma membrane^{5,8–12}. In a recent model of transepithelial sodium transport in which a regulatory role for calcium ions was incorporated, it was assumed that cytosolic calcium ion activity of epithelial cells is similar to that of excitable cells¹³. However, no direct measurements of cytosolic calcium ion activity in epithelial cells in normal transporting conditions have yet been made. We report here the direct measurement with Ca^{2+} -selective microelectrodes of cytosolic calcium ion activity in proximal tubular cells of *Necturus* kidney. The results indicate that cytosolic calcium ion activity in these cells is $\sim 10^{-7}$ M, and are consistent with the view that a Na–Ca exchange system, located at the basolateral cell membrane, plays a role in maintenance of low cytosolic calcium ion activity.

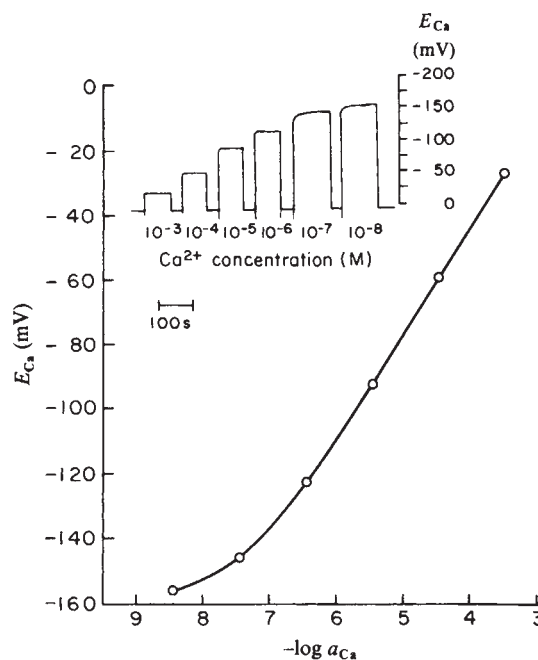


Fig. 1 Inset, microelectrode potentials measured at different calcium concentrations. Response times of the microelectrodes (time to reach a stable potential) were in the range of several seconds to one min. Main figure, microelectrode potentials plotted against $-\log a_{\text{Ca}}$. Solutions of 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , 10^{-4} and 10^{-3} M Ca^{2+} concentration were used to calibrate the microelectrode. Those containing 10^{-8} , 10^{-7} , 10^{-6} and 10^{-5} M Ca^{2+} were made from a Ca^{2+} buffer containing EGTA. The Ca^{2+} concentrations of the solutions were calculated with the apparent stability constant of $4.90 \times 10^6 \text{ M}^{-1}$ for Ca-EGTA at pH 7.0. The solutions containing 10^{-4} and 10^{-3} M Ca^{2+} were made by dilution of 0.1 M CaCl_2 solution. All the solutions contained 100 mM K^+ and 1 mM Mg^{2+} , and had a pH of 7.0. The solutions have the same ionic strength, and hence probably the same activity coefficient of Ca^{2+} . In this study, the Ca^{2+} activity coefficient of 0.35, calculated by the extended Debye-Huckel equation²⁸ was used to obtain the calibration curve shown in the main part of the figure. This procedure is justified because calculated and measured Ca^{2+} activity coefficients at various ionic strengths are in reasonably good agreement^{29,30}. The microelectrode potentials were not affected by pH changes in the range 7–8. In this study, the results are presented as activities, rather than Ca^{2+} concentrations, because: (1) many cell processes respond to ionic activities; (2) the microelectrodes measure potentials related to ionic activities; (3) expressing the results as ion concentration assumes that the activity coefficient of Ca^{2+} in the cytosol is equal to that of Ca^{2+} in the calibration solutions.

Experiments were performed on proximal tubules of doubly perfused kidneys of *Necturus maculosus*^{14,15} using Ca^{2+} -selective microelectrodes. This preparation was chosen because of the relatively large size of the epithelial cells ($\sim 20 \mu\text{m}$ in diameter) and because the transport properties of this nephron segment have been reasonably well characterized^{16,17}. Recently, Ca^{2+} -selective microelectrodes have been developed^{18–20}, which make measurements of cytosolic Ca^{2+} activities feasible. Ca^{2+} -selective microelectrodes were made employing a Ca^{2+} -selective liquid composed of 10% neutral ligand (*N*, *N'*-di(11-ethoxycarbonyl)undecyl-*N*, *N'* 4, 5-tetramethyl-3, 6-dioxaoctane diacid diamide), 1% sodium tetraphenylborate and 89% *o*-NPOE²¹. Glass micropipettes with tip diameters of $\sim 1 \mu\text{m}$ or less were pulled from borosilicate glass capillaries (Corning Glass Works, code 7740). The inner surface of the micropipettes was silanized by exposure to dichloro-dimethylsilane gas. The micropipettes were first filled with 100 mM CaCl_2 solution; the Ca^{2+} -selective liquid was drawn up to 400–800 μm from the tip. Each microelectrode was calibrated before and after use by the procedure illustrated in Fig. 1. The inset shows the potential recordings measured in calibration solutions containing calcium ion concentrations of 10^{-8} – 10^{-3} M (see

Fig. 1 legend). At calcium levels $>10^{-6}$ M the potential changes corresponded to those predicted by the Nernst equation—a 29.6 mV change for a 10-fold change in calcium ion concentration. However, at calcium ion concentrations between 10^{-6} and 10^{-7} M, the potential changes varied from 10 to 25 mV; and between 10^{-7} and 10^{-8} M, from 5 to 15 mV. (The microelectrode characteristics have been considerably improved since our initial work²².) In the main part of Fig. 1, the potentials recorded by the microelectrode are shown plotted against calcium ion activities, using an activity coefficient of 0.35 (see legend). Such a calibration curve was constructed for each electrode for the determination of intra- or extracellular calcium ion activities. Selectivity coefficients (k_{CaK}) of the microelectrodes against K^+ were in the range 1×10^{-6} – 7×10^{-6} . Selectivity coefficients (k_{CaNa}) of the microelectrodes against Na^+ were in a range similar to or slightly greater than k_{CaK} . Considering the low cytosolic Na ion activities of *Necturus* proximal tubular cells²³, cytosolic Ca^{2+} activity measurements will not be significantly affected by changes in cytosolic Na^+ activity. (Detailed properties of the microelectrodes will be published elsewhere; C.O.L., in preparation.)

Figure 2 illustrates the measurement of basolateral cell membrane potential, V_M , and cytosolic calcium ion activity, a_{Ca}^i , in a proximal tubular cell of *Necturus* kidney. An epithelial cell was first impaled with a Ca^{2+} -selective microelectrode (see lower trace). When the intracellular potential recorded with this microelectrode had reached a stable value, a second cell of the same tubule was impaled with a conventional microelectrode (see upper trace). On this impalement, the potential recorded by the Ca^{2+} -selective microelectrode, E_{Ca} , fell by an amount equal to the peritubular cell membrane potential, and then reached another stable value. The rapid change in E_{Ca} represents the electronic subtraction of V_M from E_{Ca} . The E_{Ca} following the subtraction represents the cytosolic calcium ion activity. In 11 successful measurements on seven kidneys (out of a total of 52 experiments), the cytosolic calcium ion activity of *Necturus* proximal tubular cells averaged 116 ± 32 nM (mean $\pm$ s.d.), and the peritubular membrane potential was -56.6 ± 4.0 mV. Assuming that the activity coefficient of cytosolic Ca^{2+} is the same as that calculated for extracellular fluid (0.34), the cytosolic calcium ion activity of 116 nM is equivalent to a cytosolic free calcium ion concentration of 341 nM. (This assumption is justified if the cytosolic ionic strength is not too

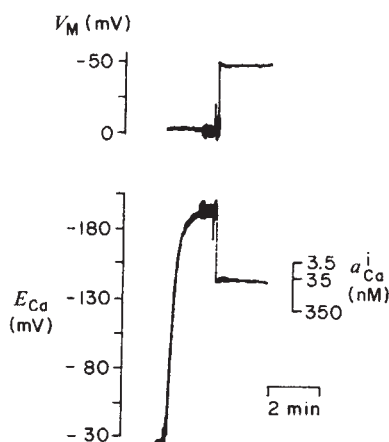


Fig. 2 Potential recordings obtained with a conventional microelectrode (upper trace) and a Ca^{2+} -selective microelectrode (lower trace) for determination of cytosolic Ca^{2+} activity. Experiments were performed on cells of proximal tubules of doubly perfused kidneys of *N. maculosus*. Both the aorta and the renal portal vein were perfused with a modified Ringer's solution²⁵ containing 100 mM Na^+ . The kidney was also superfused with the same solution. The perfusion fluid was saturated with a gas mixture of 99% O_2 and 1% CO_2 to achieve a pH of 7.5–7.6. All experiments were carried out at room temperature (22–23 °C). Cells of the same tubule were impaled with the conventional microelectrode and the Ca^{2+} -selective microelectrode.

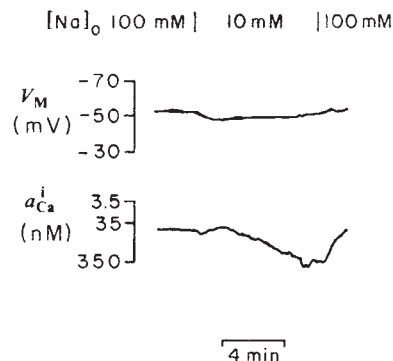


Fig. 3 Recordings of simultaneous measurements of peritubular cell membrane potential (V_M) and cytosolic calcium ion activity (a_{Ca}^i) during alteration of Na^+ concentrations in peritubular fluid. The low $[Na]_o$ solution was made by equimolar substitution of choline chloride for sodium chloride. The extracellular Ca^{2+} concentration was kept constant at 1.8 mM.

different from that of the extracellular fluid.) The value of 341 nM for cytosolic calcium ion concentration is in a range similar to that previously reported for other cell types²⁴.

To measure the electrochemical gradient for calcium reabsorption by proximal tubular epithelium, calcium ion activities were measured in tubular and peritubular fluid of perfused kidneys. In 17 measurements on five kidneys, Ca^{2+} activity averaged 0.48 ± 0.07 mM in tubular fluid, and 0.61 ± 0.04 mM in peritubular fluid ($P < 0.001$). In these tubules, the mean trans-epithelial potential difference was -5.6 ± 1.2 mV, lumen negative. Net fluid reabsorption was measured in separate experiments by the split-droplet technique²⁵; the half time of reabsorption averaged 30.9 ± 10 min ($n = 7$), a value comparable to that obtained by previous investigators²⁵. These results indicate that, in the process of calcium reabsorption, calcium ions may enter the cell passively across the luminal membrane, but must be extruded actively against a steep electrochemical gradient across the peritubular cell membrane.

Figure 3 shows simultaneous measurements of the basolateral membrane potential, and cytosolic calcium ion activity, in the control condition, and during reduction and subsequent restoration of the Na^+ concentration, $[Na]_o$, in the peritubular fluid. When $[Na]_o$ was reduced from 100 to 10 mM, the membrane potential depolarized by several millivolts. After the reduction of $[Na]_o$, cytosolic calcium ion activity increased slowly and reached a relatively stable level after 5 to 6 min. In eight successful measurements (out of 30 experiments), cytosolic calcium ion activity averaged 371 ± 59 nM ($P < 0.001$) 6 min after the change in $[Na]_o$. In four experiments it was possible to extend the recording into the recovery period following restoration of normal $[Na]_o$. In these experiments, the membrane potential and cytosolic calcium ion activity returned to levels similar to the initial value; cytosolic calcium in activity averaged 376 ± 78 nM ($\pm$ s.d.) 6 min after lowering $[Na]_o$ and had recovered to a mean value of 106 ± 22 nM ($\pm$ s.d.; $P < 0.001$) 2 to 3 min after restoring $[Na]_o$ to 100 mM. In individual impalements after restoration of normal $[Na]_o$, cytosolic calcium ion activities did not differ significantly from those in the initial control period; in the control period, cytosolic calcium ion activity averaged 115 ± 17 ($\pm$ s.d.; $n = 6$), and after restoration of normal $[Na]_o$, 118 ± 47 ($n = 5$; P , not significant).

These results are consistent with the hypothesis that a Na–Ca exchange system operates across the peritubular cell membrane and plays a significant role in the maintenance of low cytosolic calcium ion activity in proximal tubular cells of *Necturus* kidney. These findings indirectly support the view²² that the reported reduction in Na efflux from the lumen of isolated perfused rabbit proximal tubules in response to a decrease in contraluminal sodium concentration is associated with an increase in cytosolic calcium ion activity as previously postulated^{8,13,26}. Likewise an elevation of cytosolic calcium ion levels may be causally related

to the inhibition of net transport of sodium and of the hydro-osmotic response to vasopressin, observed in amphibian tight epithelia, on exposure to low serosal sodium concentrations^{5,12,27}. We conclude that cytosolic Ca ion activity, regulated in part through a process of Na-Ca exchange across the basolateral cell membrane, may be important in the physiological regulation of sodium and water absorption by these epithelial tissues.

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Evidence for an adaptive DNA repair pathway in CHO and human skin fibroblast cell lines

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When *Escherichia coli* is chronically exposed to very low, nontoxic doses of a monofunctional alkylating agent (notably *N*-methyl-*N'*-nitro-nitrosoguanidine, MNNG), the adaptive DNA repair pathway is induced which enables the bacteria to resist the killing and mutagenic effects of further alkylation damage¹⁻³. Mutation resistance in adapted bacteria is achieved, at least partly, by a greatly increased capacity of the cells to eliminate the minor DNA alkylation product *O*⁶-methylguanine⁴⁻⁶, which has been strongly implicated as premutagenic^{7,8} and precarcinogenic⁹. We now show that the chronic treatment of a Chinese hamster ovary (CHO) and a SV40-transformed human skin fibroblast (GM637) cell line with non-toxic levels of MNNG renders the cells resistant to the induction of sister chromatid exchange (SCE) by further alkylation damage. CHO cells also become resistant to killing (GM637 cells have not yet been tested). Having ruled out explanations such as changes in cell cycle distribution, mutagen permeability and mutagen detoxification, we conclude that resistance is probably achieved by the cells becoming more efficient at repairing alkylation damage, analogous to the adaptive response of *E. coli*¹.

The existence of an inducible error-free mammalian DNA repair pathway, like adaptation, could significantly affect our approach to assessing the mutagenic and carcinogenic risk of chemicals in our environment. Chronic treatment of rats with the alkylating agent dimethylnitrosamine (DMN) has been reported to enable the livers of these animals specifically to avoid the accumulation of *O*⁶-methylguanine^{10,11}. Paradoxically, chronic DMN treatment is much more carcinogenic to the rat liver than a single high dose¹². However, chronic DMN exposure also induces intense liver cell proliferation, which might obscure the role of the *O*⁶-methylguanine lesion in hepatocarcinogenesis¹⁰. Thus, although there seems to be a biochemical analogue of adaptation in the rat liver, its biological effects such as carcinogenesis, cell killing and mutation, are either obscured or cannot be quantified. The aim of our study was, therefore, to determine whether adaptation can be observed in established mammalian cell lines which would be potentially much more versatile than the rat liver system.

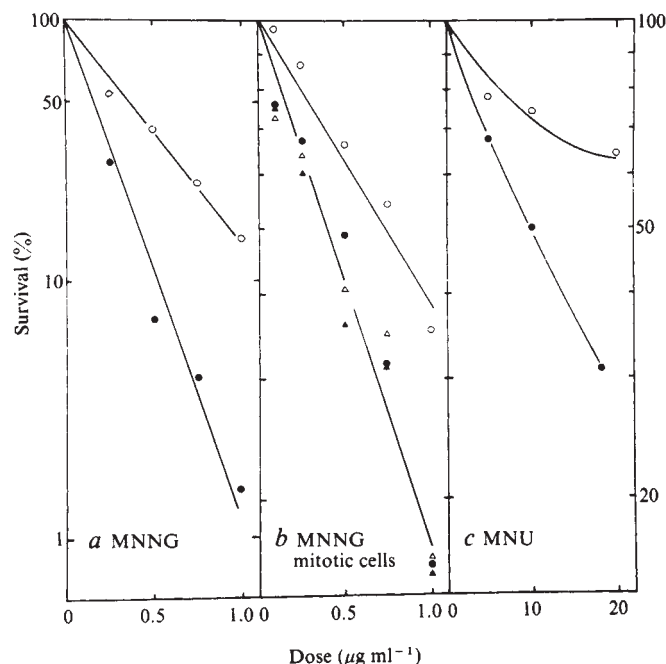


Fig. 1 The effect of MNNG pretreatment on the survival of CHO cells challenged with MNNG or MNU. Cells were grown for 48 h in Ham's F12 medium supplemented with 5% fetal calf serum, penicillin (100 U ml⁻¹), streptomycin (100 μg ml⁻¹) and L-glutamine (0.03%). Every 6 h, one set of cultures was treated with 0.01 μg ml⁻¹ MNNG. Three hours after the final pretreatment dose, pretreated (○) and control (●) cells were either trypsinized (a and c) or synchronized by mitotic shake-off (b) and then plated into 100-mm Petri dishes. Each dish was challenged with either MNNG (0-1.0 μg ml⁻¹) or MNU (0-20 μg ml⁻¹). To measure the decay of resistance, cells that had been pretreated for 48 h (Δ) were grown for a further 24 h before mitotic shake-off and challenge (▲, 72 h control). After 10 days at 37 °C, the cultures were fixed and stained. Colonies were counted in duplicate dishes.

As an initial approach we have used cell survival and SCE as our endpoints. The bacterial studies had shown that the adaptive response could only be observed when both the low dose used to adapt the cells and the high challenge dose used to estimate the degree of resistance fell within critical boundaries^{2,13}. Thus, to ensure the greatest chance of detecting adaptation in mammalian cells, a wide range of doses would have to be tested. As SCE induction has been correlated with mutation induction by simple alkylating agents¹⁴ and with the persistence of *O*⁶-methylguanine lesions in certain cell lines¹⁵, we chose this far less time-consuming assay.

MNNG has a reported half life of between 14 min (ref. 16) and 80 min (ref. 17) in cell culture medium. Consequently, the

nearest one can get to continuously exposing cells to MNNG is to deliver multiple doses over a prolonged period. (Only the most successful of several dose regimes tested will be dealt with here.) CHO cells received $0.01 \mu\text{g ml}^{-1}$ MNNG once every 6 h for 48 h. Pretreated and control cells were then challenged with various MNNG doses up to 100 times this concentration, and the resulting cell killing measured. At each challenge dose pretreated cells survived better than control cells, with about a twofold difference in the slopes of the survival curves (Fig. 1a, b). Similarly, CHO and GM637 cells that had been pretreated for 48 h and 72 h, respectively, were very resistant to the SCE-inducing effects of MNNG. Resistance to both killing (Fig. 1b) and SCE induction (Figs 2a, 3a) decayed when pretreatment was stopped.

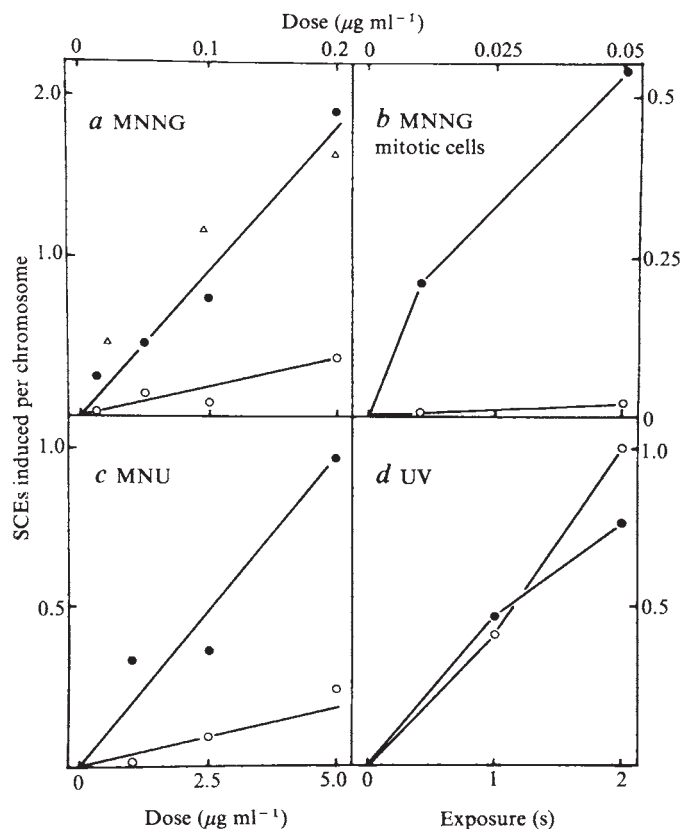


Fig. 2 The effect of MNNG pretreatment of CHO cells on the induction of SCEs. Cells were cultured as described in Fig. 1 legend. At 3 h (○) or 24 h (Δ) after the final adapting dose, pretreated (○, Δ) and control (●) cells were challenged with MNNG ($0-0.2 \mu\text{g ml}^{-1}$), MNU ($0-5 \mu\text{g ml}^{-1}$) or UV light ($0-2 \text{ s}$, fluence rate $12 \text{ erg mm}^{-2} \text{ s}^{-1}$), and then cultured for two rounds of DNA replication (26 h) in complete medium containing $10 \mu\text{M}$ bromodeoxyuridine. Colcemid ($2 \times 10^{-7} \text{ M}$) was present for the final 2 h. Mitotic cells were collected by shaking, treated with 0.075 M hypotonic KCl, then fixed in 3:1 methanol/glacial acetic acid. SCEs were differentiated by a modified fluorescence-plus-Giemsa technique²³, and at least 20 cells were counted per point. Induced SCEs were calculated by subtracting background levels ($0.35-0.5$ for control, $0.85-1.0$ for pretreated cells) from the total SCE values.

Because the sensitivity of CHO cells to MNNG fluctuates throughout the cell cycle¹⁸, a change in cell cycle distribution could generate an apparent change in the sensitivity of the overall population. However, pretreated and control CHO cells have approximately the same mitotic indices (1.25% and 1.4% , respectively) and proceed through the cell cycle at similar rates (Fig. 4a). Moreover, a cell cycle redistribution cannot account for increased MNNG resistance in CHO cells because homogeneous populations of mitotic cells also display differential sensitivity to MNNG killing (Fig. 1b) and SCE induction (Fig. 2b). An extended period in G1 might account for the observed increase in MNNG resistance¹⁹, but G1 was shown to be the

same in pretreated and control populations that had been challenged with $0.5 \mu\text{g ml}^{-1}$ MNNG (Fig. 4b).

We considered that resistance to MNNG could be achieved either by preventing alkylation from taking place or by dealing with alkylation damage more efficiently. As alkylation by MNNG is thiol stimulated, a reduction in cellular thiol levels could inhibit alkylation by this agent¹⁷. However, thiol depletion cannot explain our results because MNNG-pretreated cells were also found to be resistant to two alkylating agents which are unaffected by cellular thiol levels²⁰; CHO cells were resistant to killing (Fig. 1c) and SCE induction (Fig. 2c) by methyl-nitrosourea (MNU) and GM637 cells were resistant to SCE induction by MNU (Fig. 3b) and ethylnitrosourea (ENU) (Fig. 3c). Moreover, pretreatment does not alter the permeability of the cells to mutagen, because, for both CHO and GM637, the DNA purified from control and pretreated cultures that had been exposed to $2.5 \mu\text{g ml}^{-1}$ ^3H -MNU (120 Ci mol^{-1}) had similar specific activities. We therefore deduce that chronic MNNG exposure enables both CHO and GM637 cells to handle alkylation damage somewhat more efficiently and so become resistant to alkylating agents. This resistance seems to be specific for alkylation damage because MNNG-pretreated cells are not resistant to the induction of SCE by UV light (Figs 2d, 3d).

Thus, the response of two mammalian cell lines during continuous low-dose exposure to the carcinogen MNNG has, so far, exactly paralleled *E. coli* adaptation: a treatment which neither kills cells nor disturbs cell growth induces a resistance to alkylation damage, but not UV damage which decays once treatment is stopped. Furthermore the induced resistance in

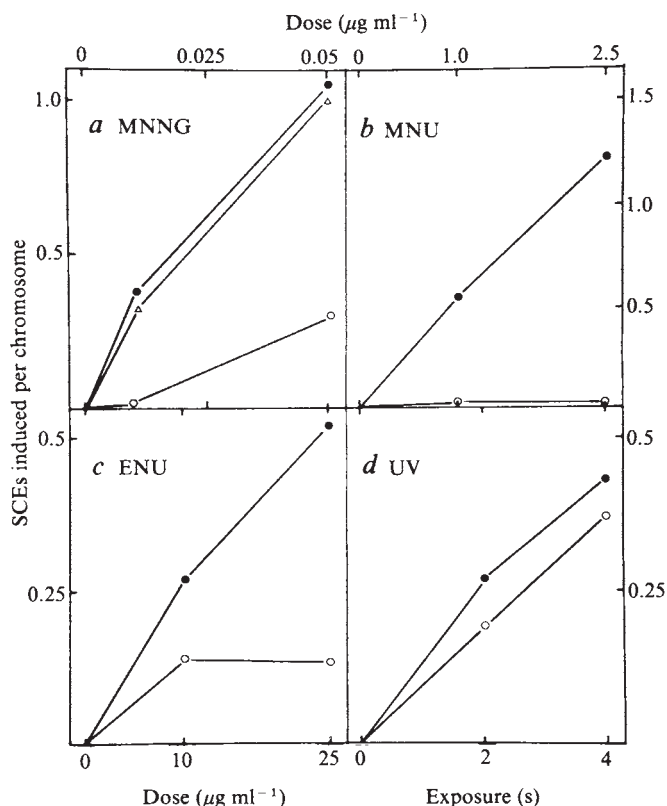


Fig. 3 The effect of MNNG pretreatment of GM637 cells on the induction of SCEs. GM637 cells were grown in minimum essential medium supplemented with 15% fetal calf serum, penicillin (100 U ml^{-1}), streptomycin ($100 \mu\text{g ml}^{-1}$), and L-glutamine (0.03%). Cells were treated and analysed as described in Figs 1 and 2 legends, except that the pretreatment regime lasted 72 h. The background SCE values were 0.26 SCEs per chromosome for controls (●) and 0.34 SCEs per chromosome for pretreated cells (○, Δ). Recovery (Δ) was measured 1 week after the final pretreatment.

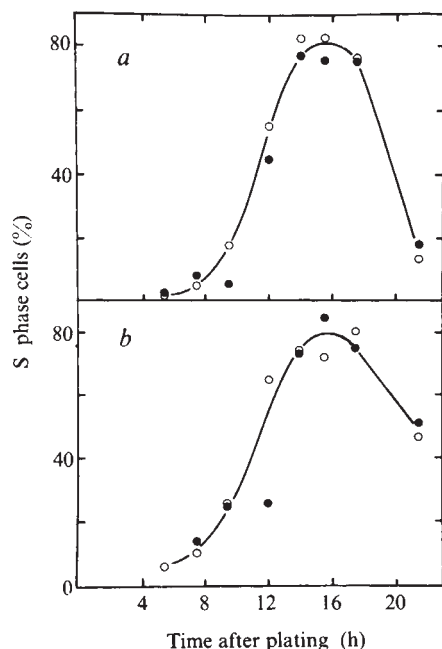


Fig. 4 The effect of MNNG pretreatment on the onset of DNA replication in synchronized CHO cells. Cells were pretreated as described in Fig. 1 legend. Three hours after the final adapting dose, pretreated (○) and control (●) cells were collected by mitotic shake-off (the mitotic index was about 0.95). Half of each group of cells were challenged with $0.5 \mu\text{g ml}^{-1}$ MNNG (b), the other half remained unchallenged (a). The cells were plated into tissue culture slide chambers at a density of 10^3 cells per cm^2 . At various times after plating, one set of slides was pulse labelled for 20 min with $10 \mu\text{Ci ml}^{-1}$ ^3H -thymidine, then washed and fixed for autoradiography. For each data point 150–350 cells were counted.

mammalian cells becomes saturated at high challenge doses (GM637, Fig. 3a; CHO data not shown) with the same biphasic kinetics seen in bacteria¹³. It is improbable that our results could be attributed to the inducible error-prone DNA repair pathway reported to exist in mammalian cells because this pathway can act on UV damage^{21,22}. However, it is not yet known whether the induced resistance to the killing and SCE-inducing effects of alkylating agents is accompanied by resistance to their mutagenic effects.

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Ca^{2+} -calmodulin-dependent phosphorylation and platelet secretion

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Protein phosphorylation may play a critical role in stimulus-coupled secretion of platelets. Some platelet proteins become phosphorylated on exposure to agents such as thrombin and collagen^{1–5}, and the smallest of these phosphoproteins (molecular weight 20,000), has been identified as a light chain of myosin^{6–8}. Phosphorylation of myosin light chain increases the activity of actin-activated myosin ATPase⁸ and the resultant contraction of the actomyosin presumably mediates the release reaction^{6,9}. Platelet myosin light chain kinase has been identified as a calcium-dependent enzyme requiring calmodulin for its activity^{10,11}. Calmodulin is a Ca^{2+} -binding protein with a molecular weight of ~18,000 which seems to be involved in a wide variety of cellular processes¹². Although a growing body of evidence suggests that non-muscle actomyosin, such as that isolated from platelets, is regulated by Ca^{2+} -calmodulin-dependent light chain phosphorylation, the precise relationship between the phosphorylation and the function of platelets is not clearly established. We now present pharmacological evidence that a calmodulin-mediated system, such as Ca^{2+} -dependent myosin light chain phosphorylation, also plays an important role in the phenomenon of the release reaction. *N*-(6-aminohexyl)-5-chloro-1-naphthalene-sulphonamide (W-7) (refs 13–15) is shown to bind selectively to calmodulin *in vitro* and inhibit its biological activity.

The effects of the calmodulin-interacting agent W-7 on human platelet aggregation and the ATP release induced by collagen ($2 \mu\text{g ml}^{-1}$), ADP ($2 \mu\text{M}$) and thrombin (0.1 U ml^{-1})

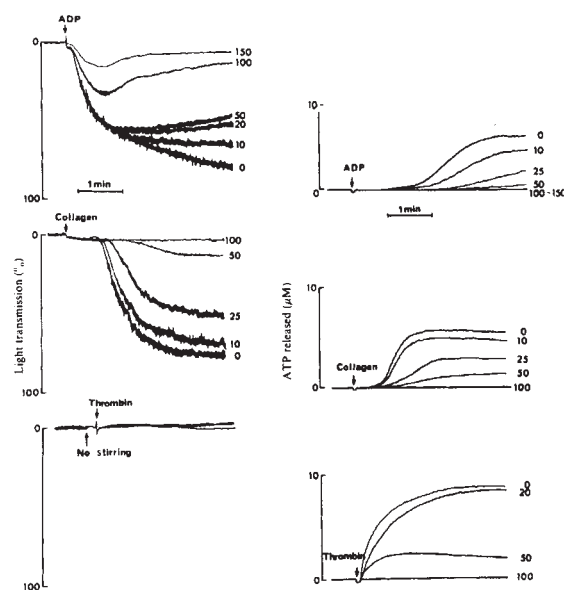


Fig. 1 Effect of W-7 on platelet aggregation and secretion induced by ADP ($2 \mu\text{M}$), collagen ($2 \mu\text{g ml}^{-1}$) or thrombin (0.1 U ml^{-1}). Secretion of ATP (right-hand traces) was measured by the firefly luminescence assay method and aggregation (left-hand traces) was determined simultaneously in the same cuvette turbidimetrically. The stirring motor was turned off to prevent aggregation in the experiment involving platelet stimulation by thrombin. The standard platelet reaction mixtures consisted of 0.4 ml of citrated platelet-rich plasma (PRP), 50 μl of Chrono-Lumi luciferase luciferin reagent, 50 μl of a dilution of W-7 (in saline), and aggregating agent. In experiments involving platelet activation by thrombin, 0.4 ml of washed platelet suspension ($4.0 \times 10^8 \text{ ml}^{-1}$) was used in place of PRP. The platelets were preincubated without (○) or with W-7 (the final concentration shown in μM by each curve) for 3 min before stimulation.

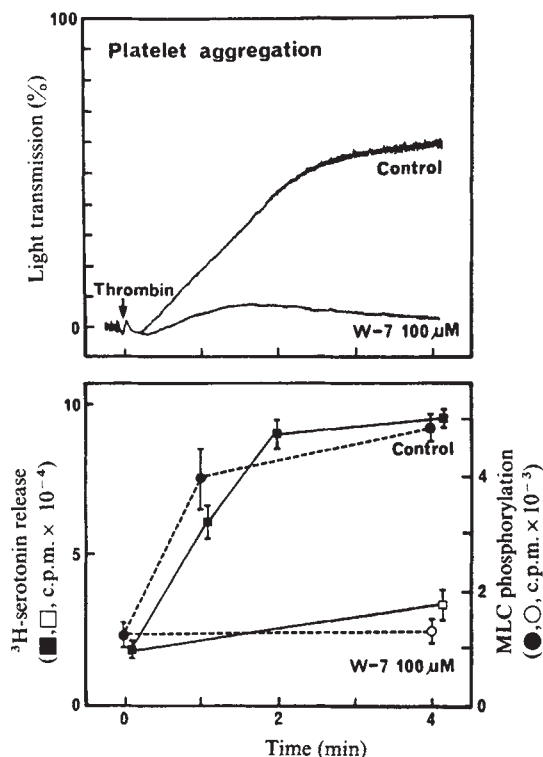


Fig. 2 Time course of platelet aggregation (top), and ^3H -serotonin release and 20,000-MW MLC phosphorylation (bottom) in response to thrombin (0.25 U ml^{-1}) and the effect of W-7 (final concentration $100 \mu\text{M}$) preincubation. Serotonin secretion and phosphorylation are shown as means $\pm$ s.e.m. ($n = 3$). ■, □, ^3H -serotonin release; ●, ○, MLC phosphorylation. Open symbols, W-7 $100 \mu\text{M}$; closed symbols, controls. **Method:** Human platelets suspension in 20 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.1% glucose, 0.35% bovine serum albumin was incubated with ^{32}P -orthophosphate at 1 mCi ml^{-1} for 30 min at 25°C . ^3H -serotonin creatinine sulphate at $1 \mu\text{Ci ml}^{-1}$ was added, and the platelets suspension was incubated for another 30 min, then spun at $600g$ for 5 min and the supernatant discarded. The pellets were resuspended in the same buffer to give a platelet count of $5\text{--}10 \times 10^8$ per ml. The platelet reaction mixtures consisted of 1.8 ml of labelled platelets suspension, 0.2 ml of saline (control) or W-7 (concentration $100 \mu\text{M}$) and thrombin (0.25 U ml^{-1}). The platelets were preincubated with saline or W-7 in the aggregometer (Rikadenki) for 3 min before stimulation with thrombin. The reaction was terminated with 1 ml of ice-cold 150 mM NaCl, 10 mM EDTA, 0.8 mM phenylmethylsulphonyl fluoride (PMSF), soybean trypsin inhibitor (0.2 mg ml^{-1}), then spun at $1,200g$ for 10 min at 4°C . Samples of supernatant fluid (1 ml) were removed immediately and transferred into liquid scintillation vials. 10 ml of ACS II (Amersham) was added and the sample counted in a Beckman scintillation counter. Platelet pellets were frozen and extracted with a high ionic strength buffer containing 0.5 M KCl, 20 mM K_2PO_4 pH 7.4, 1 mM EGTA, 0.4 mM PMSF. The insoluble material was removed by centrifugation at $150,000g$ for 20 min at 4°C . Each extract was electrophoresed on 1% SDS 10% polyacrylamide gel. The gels were stained with Coomassie brilliant blue, and photographed. ^{32}P content of 20,000-MW protein was determined by liquid scintillation counting of 2-mm gel slices.

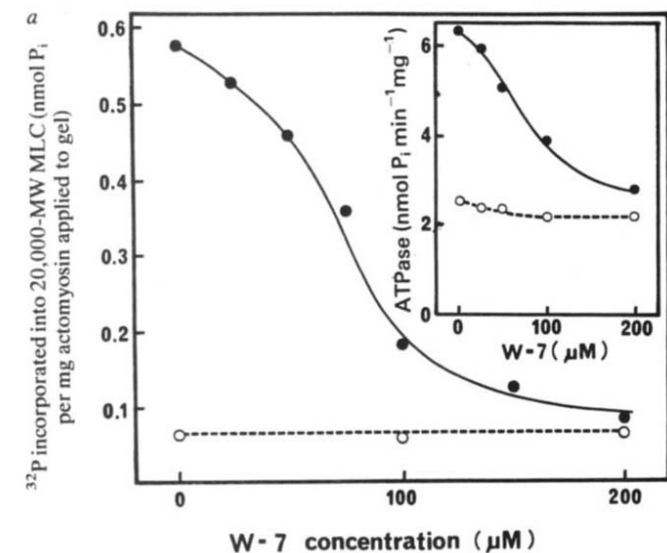
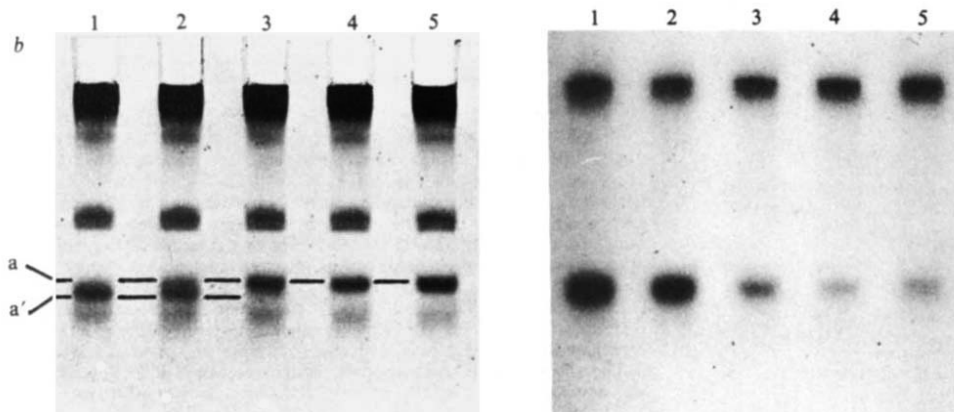


Fig. 3 *a*, Inhibition by W-7 of endogenous phosphorylation of the 20,000-MW MLC and actomyosin ATPase activity (inset) from human platelets in the presence of $100 \mu\text{M Ca}^{2+}$ (●) and in the presence of 2 mM EGTA (○). *b*, 8 M urea-gel electrophoresis (left) and its autoradiography (right) for detecting phosphorylation of the 20,000-MW MLC of platelet actomyosin. 1, Ca^{2+} $100 \mu\text{M}$; 2, Ca^{2+} $100 \mu\text{M}$ + W-7 $50 \mu\text{M}$; 3, Ca^{2+} $100 \mu\text{M}$ + W-7 $100 \mu\text{M}$; 4, Ca^{2+} $100 \mu\text{M}$ + W-7 $200 \mu\text{M}$; 5, EGTA 2 μM . *a*, The unphosphorylated 20,000-MW MLC; *a'*, the phosphorylated 20,000-MW MLC. **Phosphorylation analyses:** The reactions were carried out at 25°C for 10 min in 200 μl of reaction mixture containing 20 mM Tris-HCl pH 7.5, 125 mM KCl, 10 mM MgCl_2 , 0.5 mM $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (2 μCi per tube), platelet actomyosin (1.5 mg ml^{-1}), various concentrations of W-7, and $100 \mu\text{M CaCl}_2$ or 2 mM EGTA. The reactions were initiated by the addition of ATP and terminated by the addition of 20 μl of 5% SDS containing 0.1 M 2-mercaptoethanol and 20% sucrose. *a*, The solution containing 60 μg protein was applied to SDS-polyacrylamide gel and electrophoresis was carried out using 10% acrylamide and 100 mM sodium phosphate buffer (pH 7.2). The gels were then stained with Coomassie brilliant blue, photographed and sliced into 2-mm portions from which the radioactivity was eluted. ^{32}P radioactivity was analysed by liquid scintillation spectrometer. *b*, Urea polyacrylamide slab gel electrophoresis was carried out by the method of Perrie *et al.*¹⁸, using 10% polyacrylamide, 8 M urea, and 25 mM Tris-150 mM glycine buffer, pH 8.6. Samples of 60 μg of protein were applied to the gel. The gel was run, stained, destained and prepared for autoradiography. **ATPase assay:** ATPase activity was determined in 600 μl of medium containing 50 mM Tris-malate buffer pH 7.0, 50 mM KCl, 10 mM MgCl_2 , 1 mM ATP, platelet actomyosin (0.4 mg ml^{-1}), various concentrations of W-7, and either 2 mM EGTA or $100 \mu\text{M CaCl}_2$. Incubations were carried out for 30 min at 35°C . The reaction was stopped by the addition of trichloroacetic acid to a final concentration of 10%. Inorganic phosphate was measured by the method of Allen²¹.



were studied in a Model 400 Lumi-aggregometer (Chrono-Log.), which simultaneously monitors aggregation and release of ATP^{16,17}. A typical result is shown in Fig. 1. W-7 inhibited dose-dependently collagen-induced ATP release and aggregation. Two distinct phases of ADP-induced aggregation were evident and the inhibition by W-7 of the first phase was weaker than that of the second. The second phase of aggregation associated with ATP release was inhibited dose-dependently by W-7. Similar results were obtained when aggregation was induced by adrenaline, arachidonic acid or thrombin. When thrombin was added to the platelet suspension without stirring, ATP release occurred but there was no aggregation (Fig. 1). W-7 inhibited dose-dependently this thrombin-induced secretion of ATP, suggesting that the drug also inhibits stimulus-coupled secretion processes in platelets. W-7 had no effect on thrombin hydrolytic activity at concentrations as high as 200 μ M.

To examine the effect of W-7 on protein phosphorylation in platelet aggregation and the release reaction, protein phosphorylation in platelets was measured by prelabelling with ³²P-orthophosphate and ³H-serotonin and treating the stirred suspension of platelets with thrombin (0.25 U ml⁻¹). The platelet pellet formed after thrombin treatment was used for extraction of phosphorylated protein with a high ionic strength buffer, as described by Daniel *et al.*⁶, and the supernatant was used for the assay of serotonin. Thrombin treatment of intact ³²PO₄-loaded platelets resulted in a four- to fivefold increase in phosphorylation of the 20,000-MW protein, when a high ionic strength extract was directly electrophoresed on SDS-polyacrylamide gel. The pattern of ³²P incorporation was similar to that described by Daniel *et al.*⁶. This 20,000-MW phosphoprotein was identified as the myosin light chain (MLC) by 8 M urea slab polyacrylamide gel electrophoresis¹⁸. The phosphorylated MLC migrates slightly faster than the unphosphorylated MLC in this electrophoresis system. The change in ³²P content of MLC correlated with the increased secretion of ³H-serotonin rather than aggregation, as shown in Fig. 2. W-7 (100 μ M) inhibited both the phosphorylation of 20,000-MW MLC and serotonin release from platelets.

We examined the effect of W-7 on calcium-dependent ³²P incorporation into the 20,000-MW MLC from [γ -³²P]ATP which constitutes the Ca²⁺-sensitive regulatory mechanism of actin-myosin interaction. An actomyosin-rich fraction containing endogenous myosin light chain kinase and calmodulin (~50 units per mg protein) was prepared from human platelets by a modification of the method of Hathaway and Adelstein¹¹. ³²P incorporation into the 20,000-MW MLC from [γ -³²P]ATP was observed in the presence of calcium ions (100 μ M), and addition of EGTA to 2 mM inhibited ³²P incorporation into the MLC, indicating that this phosphorylation of MLC depends on calcium ions (Fig. 3a). Increasing the concentration of W-7 inhibited selectively the Ca²⁺-dependent phosphorylation of the 20,000-MW MLC with an IC₅₀ value of 80 μ M, as shown in Fig. 3a. Moreover, W-7 produced a concentration-dependent inhibition of Ca²⁺-dependent ATPase of platelet actomyosin (IC₅₀ 100 μ M) but did not inhibit Ca²⁺-independent ATPase. Figure 3b demonstrates that the light chains of myosin have a greater electrophoretic mobility than other platelet proteins and that the Ca²⁺-dependently phosphorylated 20,000-MW MLC migrates slightly faster than unphosphorylated MLC. Dose-dependent inhibition by W-7 of Ca²⁺-dependent MLC phosphorylation was also demonstrated by using this urea gel electrophoretic analysis.

Calmodulin interacting agents such as W-7, N²-dansyl-L-arginine-4-*t*-butylpiperidine amide (No. 233) and chlorpromazine inhibit Ca²⁺-calmodulin-dependent enzymes, for example, myosin light chain kinase^{14,15}, cyclic nucleotide phosphodiesterase^{13,19} and erythrocyte Ca²⁺, Mg²⁺-ATPase²⁰. The pharmacological effects of calmodulin-interacting agents on platelets result from a combination of several types of molecular interactions and cannot be explained by a single mechanism. However, one of the important effects of calmodulin-interacting agents is the inhibition of calmodulin-dependent MLC phos-

phorylation. Our findings indicate that there is a calmodulin-mediated regulatory system in platelets similar to that in smooth muscle tissue. The calmodulin-interacting agent W-7 could be of interest clinically as it combines antithrombotic activity with vascular relaxant activity.

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Optical properties of single elastin fibres indicate random protein conformation

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In describing the properties of fibrous proteins it has been common practice to attribute the macroscopic mechanical properties to the organization at the molecular level. Hence, the high tensile stiffness of collagen and silk has been viewed as an inevitable consequence of their crystalline structure. For elastin, however, there has been considerable controversy and confusion in assigning a conformation to this rubber-like protein. The mechanical and thermodynamic properties of elastin are consistent with the kinetic theory of rubber elasticity¹⁻⁵, and this theory is based on an isotropic network of kinetically free, random-coil molecules. In contrast, numerous electron microscope studies of negatively stained elastins obtained by auto-clave and alkali purification, as well as coacervates of the various soluble elastins, reveal a highly ordered (anisotropic) structure, consisting of 3- to 5-nm filaments that apparently run parallel to the long axis of elastin fibres⁶⁻¹¹. These filaments have been accepted as evidence for an anisotropic molecular structure in elastin that is dramatically different from the random network of the kinetic theory. We have now used polarized light microscopy to distinguish these two types of structure. We find that both purified and unpurified, water-swollen, single elastin fibres are optically isotropic, in agreement with the predictions of the kinetic theory of rubber elasticity.

Polarized light microscopy is a useful technique because unlike electron microscopy it does not require any physical modification of the protein structure, and thus it reduces the chance of procedural artefacts. Further, the technique allows the evaluation of structure at two levels of organization: (1) at the molecular level as reflected by the intrinsic birefringence, and (2) at the submicroscopic level (that is, at the level of the 3- to 5-nm filaments) as indicated by the form birefringence. A

general discussion of these types of birefringence is given by Bennet¹².

If elastin is totally isotropic, then this should result in a lack of both intrinsic and form birefringence. On the other hand, if elastin consists of isotropic filaments at the submicroscopic level, then it should exhibit an observable form birefringence. Finally, if these filaments are anisotropic at the molecular level, as is proposed in the anisotropic model of Urry¹³, then there should be an intrinsic birefringence as well as a form birefringence. The determination of birefringence for test samples that are swollen in media of differing refractive index allows one to distinguish and quantify form and intrinsic birefringence¹². Similar studies on collagen¹⁴ and chitin¹⁵ attest to the reliability of this type of analysis.

Single 6- to 8- μm elastin fibres were isolated from untreated bovine ligamentum nuchae and from ligament that had been purified by repeated autoclaving¹⁶. The fibres were viewed between crossed polarizers on a Wild M-21 polarizing microscope, and the retardation was determined at 546 nm using a Zeiss 1/30 wavelength rotary compensator. Birefringence was calculated by dividing the retardation at the centre of the fibre by the fibre diameter. The form birefringence curves were obtained from measurements on single elastin fibres and rat-tail collagen fibres swollen in liquids of known refractive index.

Both purified and unpurified single elastin fibres show an apparent positive birefringence of about 2×10^{-4} when swollen in distilled water ($n_m = 1.33$; see Fig. 1, solid square). The fact that autoclaved fibres show the same optical properties as unpurified ones argues strongly against the creation of any structural artefacts by this purification procedure. To put this low value of birefringence into perspective, curve *a* in Fig. 1 shows the birefringence expected for a system of parallel, isotropic cylindrical fibres swollen in a fluid medium of different refractive index, as predicted by an approximation of the Weiner equation derived by Stokes¹⁷. The volume fraction of the fibres

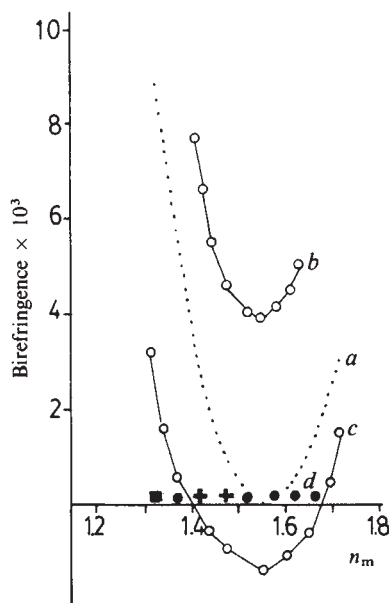


Fig. 1 (■) Birefringence of single, water-swollen elastin fibres, 2.5×10^{-4} . (●) Birefringence values for dry single elastin fibres immersed in organic liquids of different refractive index varying from $n_m = 1.36$ to 1.65. (+) Birefringence values measured for single elastin fibres swollen in ethylene glycol ($n_m = 1.43$) and glycerol ($n_m = 1.47$). Elastin swollen in these liquids retains its elastic properties. *Curve a*, Theoretical form birefringence for a system of parallel isotropic cylinders¹⁷: $B_F = (n_t - n_m)^2 v_t (1 - v_t) / \bar{n}$, where B_F is the form birefringence, n_t and n_m are the refractive index of the fibre (1.55) and the medium (1.33 for water) respectively, with $\bar{n}$ being the value of their mean. v_t is the volume fraction of the fibres (0.65) (ref. 4). *Curve b*, Rat-tail tendon collagen fibres. *Curve c*, tannic acid-fixed collagen fibres¹⁴.

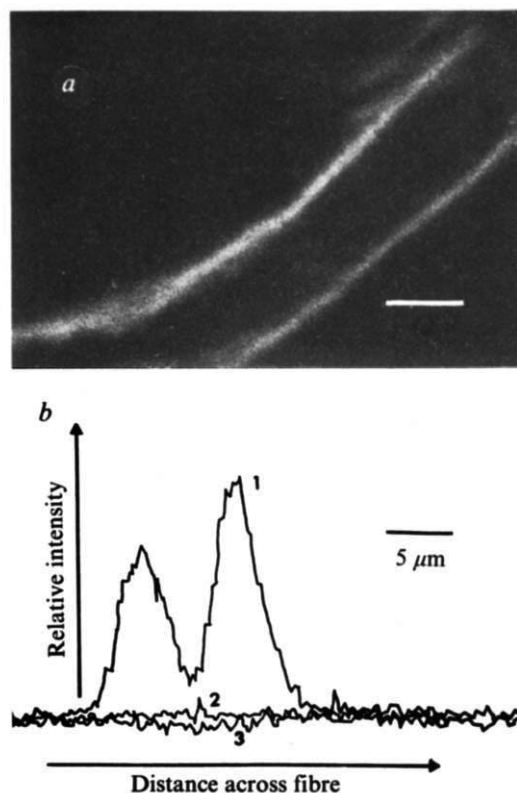


Fig. 2 *a*, Single elastin fibre ($n_t = 1.55$) in water ($n_m = 1.33$) photographed between crossed polarizers. Scale bar, 5 μm . *b*, Typical densitometer tracings for negatives of: 1, single elastin fibre in water; 2, water-swollen elastin fibre in immersion oil ($n = 1.52$); 3, control tracing of blank field.

($v_t = 0.65$) used in this calculation has been selected to reflect the known water content of elastin and thus to provide a reasonable description of elastin structure if protein filaments separated by watery spaces actually exist. Clearly the birefringence of water-swollen elastin is many times smaller than the birefringence expected for a system of isotropic filaments.

If the small residual birefringence is actually inherent to the fibre, then one would expect the retardation to be proportional to the optical path length through the fibre and should therefore be highest at the centre of the fibre. Figure 2*a* shows a water-swollen elastin fibre (refractive index of elastin, $n_t = 1.55$; refractive index of water, $n_m = 1.33$) between crossed polarizers. It is clear that the retardation, as indicated by the intensity, is greatest at the edges of the fibre. This suggests that the apparent birefringence at the centre of the fibre is not a property of the fibre itself, but results from the refractive index difference at the fibre-water interface. When this refractive index difference is reduced by suspending water swollen elastin fibres in immersion oil ($n = 1.52$) this apparent retardation virtually disappears (see the densitometer tracings in Fig. 2*b*). In fact the intensity (retardation) is indistinguishable from the background, suggesting that the true birefringence of elastin is actually zero or at least immeasurably small. We believe that this anomalous retardation created by the water-fibre interface is what has been measured by other workers⁷ who have reported very high birefringence values for water-swollen elastin.

Although elastin appears to be completely isotropic, the possibility exists that the zero total birefringence is due to the summing of a positive form birefringence that is just equal to a negative intrinsic birefringence. This possibility is demonstrated in Fig. 1 for collagen fibres and tannic acid-fixed collagen fibres¹⁴. Collagen fibres, which are known to be anisotropic at both the molecular and the submicroscopic levels, show both positive form and positive intrinsic birefringence (Fig. 1, curve *b*). Tannic acid fixation reverses the sign of the intrinsic bire-

fringe, and as a result of this, tannic acid-fixed fibres 'appear' to be isotropic (that is, show zero total birefringence) at $n_m = 1.40$ and $n_m = 1.67$ (Fig. 1, curve *c*). That the lack of birefringence in single elastin fibres does not occur as a result of this phenomenon is confirmed by the fact that elastin fibres swollen in a variety of solvents over a broad range of n_m show the same small birefringence as water-swollen fibres (Fig. 1, curve *d*). Thus, elastin shows neither intrinsic nor form birefringence, indicating an isotropic structure at both the molecular and the submicroscopic levels.

In conclusion, the extremely low residual birefringence values and the absence of any form birefringence leads us to believe that elastin does in fact possess a random conformation at the molecular level, similar to other known elastomeric materials. Although these data do not preclude the possibility that elastin contains randomly oriented filaments, there is clearly no evidence for the existence of the highly ordered, 3- to 5-nm filaments visualized by negative staining techniques in the elec-

tron microscope. We suspect that the filamentous organization seen for elastin may well be the result of drying elastin in the presence of the heavy-metal salts used for negative staining. It is also possible, however, that these filaments are cellulose contaminants, as suggested previously¹⁸⁻²⁰. It is our opinion that the 120-nm subfibres which have been observed in the scanning electron microscope²¹ are large enough to accommodate an isotropic random network structure, and thus are not in conflict with the idea of elastin being a typical kinetic rubber. However, the lack of form birefringence for elastin fibres suggests that these subfibres may only be due to a rough surface texture of the 6- μ m fibres and may not extend through the entire thickness of the fibre. This interpretation is consistent with the appearance of elastin fibres seen in cross-section in the transmission electron microscope²².

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Motile flagellar axonemes with a 9+1 microtubule configuration

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Electron microscope (EM) studies of the eukaryotic flagellum reveal that the organelle contains a 9+2 arrangement of microtubules, the axoneme, with nine doublets surrounding two singlets enveloped by a membrane which is continuous with that of the cell; various linkages and projections are associated with the microtubules¹. Strong experimental evidence supports the idea that the forces required for bend formation on eukaryotic flagella are derived from active relative sliding of the peripheral doublets^{2,3}. Dynein arms, which project from each peripheral microtubule and possess ATPase activity, interact with a neighbouring doublet and undergo conformational changes which induce sliding^{4,5}. To form and propagate coordinated bends along a flagellum the sliding must be resisted in a controlled manner by structures within the axoneme. The regulatory mechanism responsible for the control of inter-doublet sliding is not known in detail, but ultrastructural studies⁶ suggest that interactions between the radial spokes attached to each doublet and the central complex of the axoneme may be involved. We report here the treatment of flagella with a 9+2 microtubular structure from the trypanosomid flagellate *Crithidia oncopelti* to produce motile axonemes with only one central microtubule. We conclude that the complete central complex is not involved in the conversion of microtubule sliding into axonemal bending, but may be both associated with the control of wave propagation and essential for bend initiation.

Cells from a 5-6-day old culture of *Crithidia* were demembrated by exposure to detergent using a procedure similar to that described by Holwill and McGregor⁷, except that the chelating agents EGTA and EDTA were omitted from all solutions. Samples were removed from a particular preparation

at periods of 1 min, 1 h and 4 h for structural examination by electron microscopy and for motility studies following reactivation with ATP. Samples were reactivated at room temperature on a microscope slide by gently mixing a drop containing demembrated cells with a roughly equal volume of reactivating solution containing 1 mol m⁻³ ATP. Reactivated cells were examined in a Zeiss WL Research microscope with stroboscopic illumination.

Structural studies of reactivated and non-reactivated cells were undertaken after an incubation period of 1 h; because no structural differences between the two preparations were detected, subsequent samples for electron microscopy were prepared without the addition of ATP. Transverse sections of axonemes were examined at the three sampling times specified above and classified according to the pattern of microtubules remaining. Four distinct categories could be recognized: 9+2, 9+1, 9+0 and A-microtubules held together in cylindrical arrays, presumably by nexin links (Fig. 1). The proportions of axonemes falling into these classes are presented in Table 1.

We have not established unequivocally which of the two central microtubules is removed first during the extraction process, but results obtained for other organisms suggest that it is probably CM3, as this microtubule is chemically less stable than CM8 (ref. 8). Because of their importance in motility the number of dynein arms in a section was determined and expressed as a percentage of the total expected. In practice, only the outer arms were scored, as these were solubilized before the inner arms and were easier to visualize. The results of these counts are presented in Table 1.

In assessing motility, only those cells which were propagating coherent bends were scored as motile; organelles with an oar-like or a local bending movement were considered to be non-motile. The proportions of motile cells observed in a typical experiment are shown in Table 1. In all cases, wave propagation was from base to tip, with the beat pattern similar to that described by Holwill and McGregor⁷. There were no major differences between the wave shapes displayed by flagella of a given sample, as judged by visual examination of the sample, but the beat frequency of motile cells was generally observed to

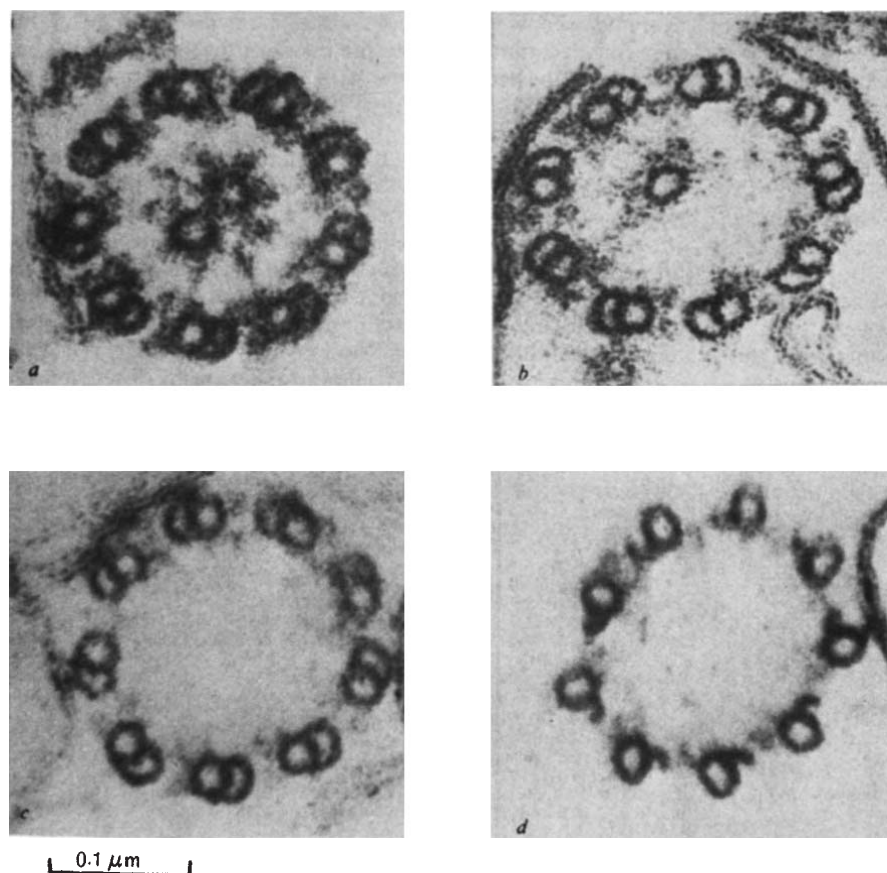


Fig. 1 Transverse sections of detergent-treated axonemes of *Crithidia oncopelti* showing the changes in microtubule arrangement which occur during degradation. *a*, Intact 9+2 axoneme; *b*, axoneme with one central microtubule missing; *c*, axoneme with both central microtubules missing; *d*, cylinder of A-microtubules. Membrane removal was performed at a ratio of 1 mg of cell protein (measured by the method of Lowry *et al.*¹⁷) to 1.0 cm³ of 0.1% detergent solution, and the demembrated cells were incubated at 0 °C. The incubation periods for the axonemes represented by the sections shown were 1 h for *a* and *b* and 4 h for *c* and *d*. In preparation for electron microscopy, cells were fixed in 2.5% glutaraldehyde in phosphate buffer at room temperature, post-fixed in 1% osmium tetroxide, dehydrated and embedded in Spurr resin.

decrease with time. A detailed study of bend patterns is planned to determine whether subtle shape changes occur which may be attributed to variations in the axonemal structure.

Of particular interest in correlating structure with motility are the results obtained after 1 h of incubation. Whereas 83.2% of the sample was motile, only 15% of the axonemes examined had a 9+2 microtubular configuration. A large percentage of the axonemes with the 9+1 structure was therefore propagating bends at this time. After 4 h, the proportion of 9+1 axonemes in a sample is similar to that after 1 h, but the percentage motility has fallen by about 70. This decrease cannot be due to the 7% reduction in the number of dynein arms present at the later time, but could be due to a loss of functional integrity in these structures.

The fact that the axonemes with a disrupted central complex are capable of propagating bends leads to the question of whether any of the central complex is necessary for wave propagation. That 9+0 flagella may have been non-motile in the present study does not mitigate against this possibility, for structures other than the central complex could have been removed, without detection, during the treatment period, and this could be responsible for the lack of movement.

Although this is the first report of an axoneme which remains

capable of activity after removal of a central microtubule, reports of undulating flagella with 9+0 (ref. 9), 6+0 (ref. 10) and 3+0 (ref. 11) microtubule configurations also indicate that central structures are not essential for movement. It may also be relevant that some insect spermatozoa have no central microtubules and yet are motile^{12,13}; however, these spermatozoa have accessory components in addition to the nine doublets so that observations on these cells do not provide strong evidence that a cylinder of doublet microtubules alone is capable of coordinated bending activity.

That the integrity of the central complex may be important for motility is indicated by experiments on *Chlamydomonas* mutants which lack one or both of the central microtubules and seem to be incapable of bend formation and propagation^{14,15}. Treatment of both mutant and wild-type flagella with trypsin, followed by ATP, induced identical disintegration processes which involved relative sliding of the peripheral microtubules, thus indicating that the dynein arms of the mutant organelles are able to generate shearing forces. Further observations which suggest that the central microtubules are necessary for bending movement have been made recently by Afzelius and Eliasson¹⁶, who reported that spermatozoa from a man suffering from situs inversus lacked one of the central microtubules of the axoneme

Table 1 Results from a typical experiment to investigate motility of demembrated flagella of *Crithidia oncopelti*

Incubation time (min)	Microtubule configurations and associated outer dynein arms (D)* (%)								Motile cells (% + s.d.)	Sample size	
	9+2	D	9+1	D	9+0	D	A-cylinders	D		No. of EM sections	No. of cells observed in light microscope
1	79.2	100	19.5	100	0.0	—	1.3	0	93.9 ± 1.2	77	412
60	15	90.5	79.3	86.6	4.7	0	0.9	0	83.2 ± 2.3	213	286
240	4.0	93.3	78	79.6	9	48.9	9	0.8	13.9 ± 2.1	90	280

* The values for the outer dynein arms are percentages of the total expected for that particular microtubule configuration.

and were immotile, and by Baccetti, Pallini, Mazzini and Ranieri (personal communication), who find that human sperm lacking both central microtubules are immotile.

The motile properties of the 9+1 axonemes of *Crithidia* reported here are not shared by axonemes with a similar structure from *Chlamydomonas* mutants, which are characterized by paralysis. A possible explanation for the different behaviour observed in the two cases is that features other than the central complex which are important for motility are affected in the mutant system. Changes in such features may be too subtle for detection by electron microscopy, as can be seen from the results presented here for axonemes at 1 and 4 h. The 9+1 cross-sections prepared from both samples appeared identical, but, when the number of other microtubule patterns are allowed for, it is clear that some of the 9+1 sections taken at 4 h are from non-motile flagella.

The data relating to microtubule arrangement presented in Table 1 can be interpreted in two ways: either (1) the percentages quoted represent the sample fraction in which each flagellum in its entirety has a particular structure, or (2) each flagellum in a sample contains a particular microtubule configuration for the appropriate percentage of its length. For example, at 1 h, interpretation (1) would have 15% of the sample with a 9+2 arrangement and 79.3% with a 9+1 arrangement, whereas according to interpretation (2), 15% and 79.3%, respectively, of each flagellum would have the 9+2 and 9+1 configurations. We have attempted to distinguish between the two alternative interpretations by electron microscopic studies of sections and of whole mounts, but our results are inconclusive. For example, serial sections taken over a portion of the axoneme 350–450 nm long (about 2% of the total axoneme length) were examined for 15 organelles with the 9+1 structure and all were found to have the same appearance.

Whichever of the two structural interpretations is correct, it is clear that coordinated waves can be propagated by an axoneme with a disrupted central complex, so that the conversion into bending moments of the shearing forces developed by the sliding peripheral microtubules is probably not achieved through interactions between the radial spokes and the central complex. This conversion could be accomplished if, as is thought to be the case, microtubules are associated with structures at the flagellar base which prevent their free relative sliding.

From a purely mechanical viewpoint, therefore, bend propagation could occur in the absence of any interaction between the peripheral and central microtubules. The interaction between these structures which seems to occur in the *in vivo* 9+2 flagellum may therefore be associated with the control of wave propagation (perhaps in determining the direction of bend propagation or the plane of bending in *Crithidia*) and not be involved directly in the mechanics of the conversion of sliding into bending.

If each axoneme contains a varying microtubule pattern (interpretation (2)), it seems reasonable to suppose that degradation of the axoneme occurs in an ordered manner, say from the flagellar tip, so that the axoneme shows progressive structural deficiency from base to tip. One possible interpretation of our results would then be that the full 9+2 complement of microtubules is required for bend initiation (as all bend propagation was from the base), but that bend propagation can be maintained by regions of the axoneme with 9+1 or, possibly, 9+0 microtubule arrangements. This situation could explain the difference in behaviour between *Crithidia* flagella and the *Chlamydomonas* or sperm mutants, which presumably have the 9+1 configuration along their entire length. On this interpretation, the results presented here suggest that a minimum length (between 4% and 15%) of the axoneme should contain the 9+2 arrangement of microtubules for successful bend initiation to occur.

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Expression of a transposable antibiotic resistance element in *Saccharomyces*

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Some eukaryotic genes can be expressed in bacteria but there are few examples of the expression of prokaryotic genes in eukaryotes¹. Antibiotic G418 is a 2-deoxystreptamine antibiotic that is structurally related to gentamicin² but has inhibitory activity against a much wider variety of pro- and eukaryotic organisms. In bacteria, resistance to G418 can be determined by several plasmid-encoded modifying enzymes³ and, in view of the broad spectrum of activity of G418, we considered that this antibiotic might be useful as a selective agent for the introduction of these antibiotic resistance genes into a eukaryotic organism such as *Saccharomyces cerevisiae*. Additional impetus for these experiments came from the knowledge that certain of the G418-resistance determinants in bacteria are carried on transposable elements⁴; a study of the properties of these elements in eukaryotes would be intriguing.

We have transformed *S. cerevisiae* spheroplasts⁵ with a mixture of DNA of the yeast *leu2* cloning vector pYE13 (ref. 6) and a colicin E1 derivative (pA043) carrying the transposable element Tn601(903)⁴. The latter encodes aminoglycoside phosphotransferase-3' (I) (APH(3')-I) that phosphorylates and inactivates a number of aminoglycoside antibiotics containing the 2-deoxystreptamine moiety, at the 3'-hydroxyl position. We chose to use co-transformation with pYE13 and pA043 because selection of the *leu*⁺ phenotype provided a control for efficiency of transformation, and in addition, we anticipated that subsequent recombination would lead to the formation of a hybrid plasmid. Following regeneration, prototrophic transformants were selected and screened for resistance to G418; the frequency of resistant transformants was about 8% of the *leu*⁺ progeny. The G418-resistant colonies were capable of growth in the presence of >1 mg ml⁻¹ antibiotic. The untransformed parent, or strains transformed with the yeast vector pYE13 alone, were sensitive to low concentrations of G418 (Fig. 1). In separate experiments we have shown that *S. cerevisiae* mutants resistant to low levels of G418 (150 µg ml⁻¹) appear spontaneously at low frequency (<10⁻⁵) and that this frequency is drastically diminished (<10⁻⁷) when one selects for higher resistance levels (500 µg ml⁻¹) of G418, as assayed by plating on media containing the antibiotic.

The presence of APH(3')-I activity in these transformants would show that the G418 resistance was due to the expression

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Table 1 Substrate specificity for the APH(3')I activity from G418-resistant yeast transformant

Antibiotic tested	c.p.m.	
	Yeast (JG13) extract	<i>E. coli</i> extract
Neomycin B	5,617 (100)	18,652 (100)
Lividomycin A	5,767 (102)	26,262 (140)
Butirosin	1,389 (25)	5,078 (27)
Tobramycin	353 (6)	30 (0)
G418	3,163 (56)	4,364 (23)

S. cerevisiae transformant JG13 was grown at 30 °C in 200 ml YEPD medium containing 200 µg ml⁻¹ G418 to a density of 7 × 10⁷ cells ml⁻¹. Cells were washed with water, resuspended in 0.7 ml 50 mM Tris-HCl, pH 7.6, 12.5 mM MgCl₂, 80 mM KCl, 1 mM dithiothreitol (DTT) buffer and disrupted by passing through a French pressure cell at 18,000 p.s.i. Cell extracts were centrifuged at 150,000 g for 2 h and the supernatant (0.5 ml) was passed through a Sephadex G-200 column (32 × 1.5 cm) using 10 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 1 mM DTT, 400 mM NaCl buffer. APH(3')-I activity eluted at a position corresponding to a protein of molecular weight ~60,000. *E. coli* KH802 carrying the plasmid pA043 was grown in 200 ml ML medium (1% tryptone, 1% NaCl, 0.5% yeast extract) to an A₅₅₀ of 0.75. Cells were centrifuged, washed with saline, resuspended in 0.7 ml 10 mM Tris-HCl, pH 7.6, 1 mM MgCl₂, 0.15 mM EDTA and then disrupted as described above. The extract was centrifuged for 2.5 h at 150,000 g and the supernatant used as a source of APH(3')-I enzyme. APH(3')-I from the yeast transformant JG13 was assayed in 102-µl incubation mixtures containing, 14 mM Tris-HCl, pH 7.5, 32 mM NH₄Cl, 323 mM NaCl, 11.5 mM MgCl₂, 0.1 mM Na₂EDTA, 1 mM DTT, 0.06 mM [γ-³²P]ATP (Amersham, specific activity 10 Ci mol⁻¹), 80 µl of the peak fraction and 20 µg of the relevant aminoglycoside antibiotic. APH(3')-I from *E. coli* was assayed in 32-µl incubation mixtures containing 9 mM Tris-HCl, pH 7.1, 6 mM MgCl₂, 53 mM NH₄Cl, 0.2 mM DTT, 0.1 mM Na₂EDTA, 0.06 mM [γ-³²P]ATP, 60 µg ml⁻¹ of the relevant antibiotic and 10 µl of *E. coli* KH802/pA043 extract. Reactions were incubated at 30 °C for 15 min and then 90- or 25-µl samples (for yeast or *E. coli* extracts, respectively) were spotted onto P-81 Whatman paper, washed with water and counted as described previously⁷.

of the Tn601(903) gene. Accordingly, cell-free extracts from sensitive and resistant transformants were prepared and tested for their content of the enzyme using standard assay procedures⁷; the extracts were passed through Sephadex G200 before assay to remove ATPase activity that may limit the extent of aminoglycoside phosphorylation. Chromatography of extracts of G418-resistant transformants gave a peak of strong phosphotransferase activity. The substrate range of this enzyme activity corresponded to that of a typical APH(3')-I (Table 1). No such enzymatic activity could be detected in extracts of untransformed controls. Thus, we conclude that the phosphotransferase encoded on the element Tn601(903) can be introduced and expressed in yeast.

As confirmation of this we have established that G418-resistant transformants contain Tn601(903) sequences. Total cellular DNA was isolated from parental and transformed yeast strains, electrophoresed in an 0.7% agarose gel and transferred to nitrocellulose sheets according to the method of Southern⁸. The transferred DNA was hybridized to ³²P-labelled λ :: Tn601 and pBR322 probe. As shown in Fig. 2a, the former probe, which has no homology with yeast, colicin E1, or pYE13 DNA, hybridizes specifically to DNA isolated from G418-resistant transformants.

Table 2 Stability of the G418-resistant phenotype in Tn601 yeast transformants

Growth medium	% No. of colonies			
	YEPD	YEPD +G418	Minimal	Minimal +Leu
YEP	100 (340)	44 (150)	52 (176)	97 (330)
YEP + G418	100 (222)	70 (156)	73 (162)	96 (216)
Minimal	100 (316)	50 (158)	62 (196)	99 (312)
Minimal + leucine	100 (386)	61 (234)	67 (250)	108 (416)

Strain JG13 was grown for 10 generations in either YEPD or YEPD + 125 µg ml⁻¹ G418 or for five generations in either minimal medium (0.76% Difco yeast nitrogen base w/o amino acids supplemented with 40 µg ml⁻¹ histidine) or minimal supplemented with 40 µg ml⁻¹ leucine. Serial dilutions of these cultures were plated onto selective media; numbers in parentheses represent total colonies scored on each medium.

The position of the sequences homologous to Tn601 in the transformants is coincident with that of sequences hybridizing to pBR322 (Fig. 2b), which suggests that the transposable element Tn601 is present in a hybrid pA043/pYE13 molecule. The intensity and the relative positions of hybridization of Tn601 and pBR322 to DNA from JG13 and JG16 suggest that the putative chimaeras in the two transformants differ.

The G418 resistance of the yeast transformants is lost on growth in the absence of antibiotic (Table 2); this loss occurs at approximately the same frequency as loss of the leu⁺ character encoded by pYE13, which suggests that *leu2* and Tn601(903) are associated. As confirmation of this, we have isolated recombinant pYE13/pA043 plasmid DNA from yeast transformants JG13 and JG16 and have used them for transformation of both *S. cerevisiae* and *Escherichia coli*, selecting either for leucine prototrophy or directly for resistance to G418. The plasmid DNAs from the two sources show differences in restriction digestion patterns, in agreement with the results of hybridization studies (A. J., unpublished observations).

The introduction of a bacterial resistance gene into yeast (as a transposable element) by direct selection of G418 resistance following transformation, raises some interesting possibilities for further study: (1) The control and expression of bacterial genes in eukaryotes; the use of the antibiotic modifying enzymes allows a simple assay for gene expression and could be used for the isolation of eukaryotic promoter sequences. (2) The expression and possible function of transposable elements: Tn5, for example, encodes aminoglycoside modification and several functions required for its own transposition⁹. It will be of interest to determine if these recombination capacities can operate in yeast and other eukaryotes. (3) G418 is an antibiotic with wide range inhibitory activity; it is a potent inhibitor of protein synthesis in cell-free extracts of bacterial, fungal, algae, plant and animal cells (S. Perzynski, unpublished). The combination of this antibiotic with any one of several bacterial resistance determinants should provide useful selection procedures for universal cloning vectors. Although, in principle, any of these

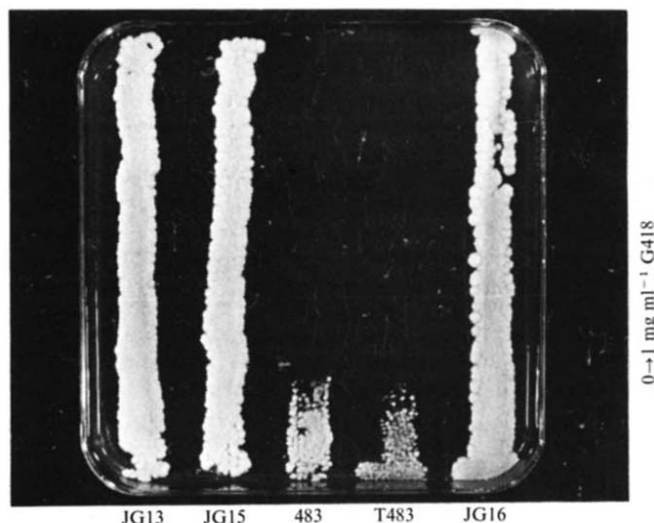


Fig. 1 Growth on G418 gradient plates of parental and transformed yeast strains. *S. cerevisiae* strain 483 (a, *leu2-3 leu2-112, his4, can1* was transformed as described by Hinnen *et al.*⁵, using a mixture of 20 µg ml⁻¹ each of pA043 (a Tn601-miniColE1 plasmid) and pYE13 (ref. 6) (a yeast-cloning vector containing the *leu2* gene), selecting for leucine prototrophy⁵. After 5 days incubation at 30 °C leu⁺ transformants were streaked on YEPD plates containing 125 µg ml⁻¹ G418 to screen for co-transformation of resistance to G418. About 8% of the leu⁺ prototrophs were resistant to the drug. To examine levels of resistance, colonies were resuspended in YEPD medium at a density of 2 × 10⁷ per ml and streaked onto an agar plate containing a gradient of antibiotic G418 from 0 to 1,000 µg ml⁻¹. JG13, JG15 and JG16 are G418-resistant co-transformants; T483 is the pYE13-transformed 483 strain and 483 is the *leu2-3* parental *S. cerevisiae* yeast strain used in the present work.

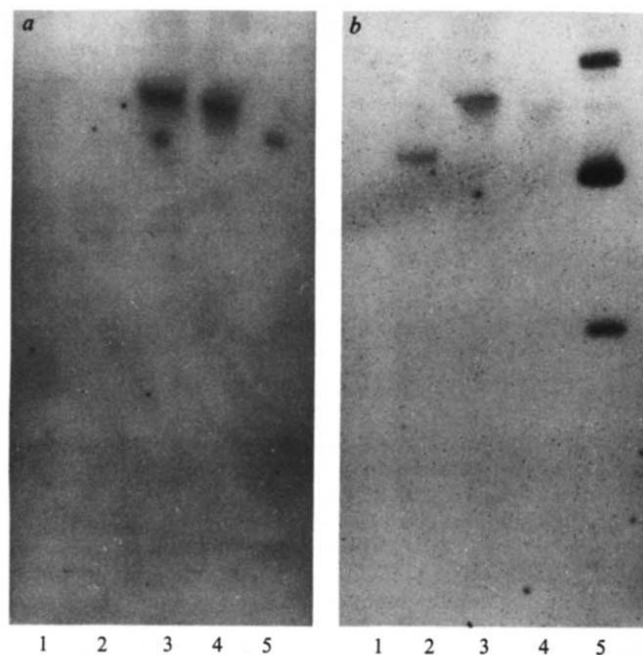


Fig. 2 Hybridization of total yeast DNA to ^{32}P -labelled (λ ::Tn601 (a) and pBR322 (b) DNA probes. Yeast strains 483 (1) and T483 (2) were grown in YEPD medium and strains JGI3 (3) and JGI6 (4) were grown in YEPD medium containing $150\text{ }\mu\text{g ml}^{-1}$ G418. DNA was extracted from yeast spheroplasts, treated with α -amylase, pronase, pancreatic RNase and extracted with chloroform/isoamyl alcohol¹⁰. About $5\text{ }\mu\text{g}$ of each DNA sample was electrophoresed on two 0.7% agarose gels; well number 5 contained $0.2\text{ }\mu\text{g}$ pYE13 DNA. DNA was transferred from the gels to nitrocellulose paper by the blotting procedure of Southern⁸ and was hybridized to ^{32}P -labelled λ ::Tn601 (a) and pBR322 (b) DNA (provided by S. Stibitz). The filters were exposed to Kodak X-ray film for a suitable period of time, developed and scanned.

determinants could be used, we have found that, in yeast, the phosphotransferase of Tn601(903) is most effective. This may reflect some regulatory sequence peculiar to this gene. The resistance genes can be added to the appropriate plasmid or viral vector by transposition or by cloning (detailed restriction endonuclease maps are known for several of the G418-resistance determinants). For example, this could allow the cloning of replication origins by linkage to the resistance determinant.

The use of G418 for cloning vector selection should eliminate the necessity for special conditions (aerobic or anaerobic growth) or specially constructed recipients for transformation (auxotrophs); thus, this antibiotic resistance should be valuable in the genetic manipulation of industrial yeast strains. A wide variety of yeast species and genera are sensitive to G418 (unpublished observations).

We thank Dr Peter Daniels (Schering Corporation) for gifts of antibiotic G418 and many related compounds, and Gary Gray, Stephen Harford and Scott Stibitz for their help and advice. Dr Takeshi Oka provided an *E. coli* strain carrying pA043 and Dr J. Hicks provided both a source of the yeast vector pYE13 and the yeast strain 483. This work was supported by grants from the NIH (AI12448) and the Comité Conjunto Hispano Norteamericano para la Cooperación Científica y Tecnológica. *Note added in proof:* Following submission of this manuscript, we were informed by C. P. Hollenberg (personal communication) that he had transformed *S. cerevisiae* with a kanamycin resistance plasmid and had obtained kanamycin-resistant colonies.

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Chelation of cadmium with BAL and DTPA in rats

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Cadmium is unique among non-essential metals in several of its toxicological effects because of its long biological half life, slow excretion and delayed action on the kidneys¹. Although cadmium poisoning in humans is uncommon, there are reports of chronic cadmium poisoning in workmen^{2–4} and also in others in certain polluted areas^{5,6}. At present there are no suitable methods either to measure the body burden of cadmium or to treat cadmium poisoning⁷. The therapeutic effects of various chelating agents including 2,3-dimercaptopropanol (BAL) and its soluble glycosides have been studied without much success in acute cadmium intoxication^{8–11}. However, those studies were done before anything was known of the specific binding of cadmium to metallothionein, an intracellular low molecular weight protein¹². The potential role of this protein in the detoxification and toxicity of metals has been reviewed recently^{13,14}. The induced synthesis of metallothionein has a marked effect on the pharmacokinetics of cadmium and other divalent metals^{15,16}. Thus it is important to consider the intracellular binding of cadmium with metallothionein in developing a suitable chelation therapy for chronic exposure to cadmium¹⁷. The *in vivo* chelation of cadmium with BAL or BAL and diethylenetriamine pentaacetic acid (DTPA) from rats exposed to cadmium is reported here.

A group of male rats (150 g) was injected intraperitoneally (i.p.) with $^{109}\text{CdCl}_2$ (1 mg Cd; $5\text{ }\mu\text{Ci}$) and kept in separate metabolic cages. Three days later they were divided into three groups and treated i.p. as follows for 5 d per week for 2 weeks: group 1 (11 rats) with BAL (50 mg per kg); group 2 (5 rats) with 50 mg each of BAL and DTPA, and group 3 (9 rats, control)

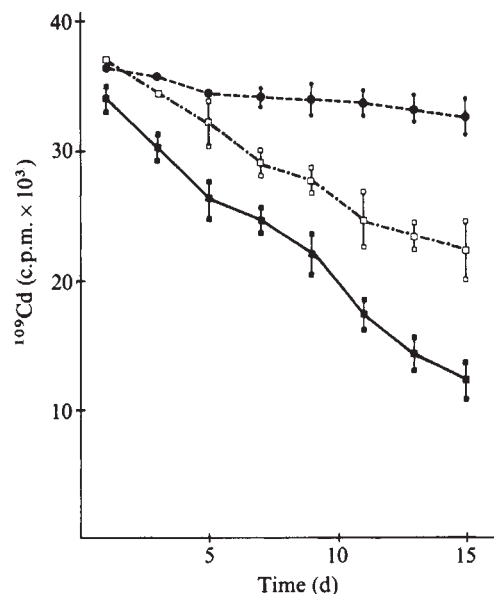


Fig. 1 Total body burden of cadmium. Rats were injected with $^{109}\text{CdCl}_2$ (1 mg per kg; $5\text{ }\mu\text{Ci}$) and 3 d later were treated i.p. with BAL (group 1, $\square$) or BAL and DTPA (group 2, $\blacksquare$) or saline (group 3, control, $\bullet$) for 2 weeks. The body burden of cadmium was determined from the whole body counting.

with normal saline. Urine and faecal samples were collected from each rat daily and the total excretion of cadmium was calculated from radioactivity. All rats were examined for ^{109}Cd radioactivity in a whole body counter¹⁸ before and during the experiment.

Injections of BAL, both alone and in combination with DTPA, decreased the total body burden of cadmium in cadmium-exposed rats (Fig. 1), which retained only 57% and 40% of initial radioactivity, respectively. The control group on the other hand, retained about 92% of the cadmium. Thus the chelating agents effectively removed cadmium from the body when treatment started 3 days after injection of cadmium. In our previous *in situ* studies¹⁷, BAL was the only chelating agent able to remove cadmium through the bile after the metal was bound to metallothionein. Compounds such as 1,3-dimercaptopropanol and dimercaptosulphonic acid, which are structurally similar to BAL, had no effect. Therefore structural features, such as adjacent sulphhydryl groups and lipophilic properties of chelating agents, are important for the chelation of cadmium from metallothionein *in vivo*.

Analysis of excreta for radioactive cadmium showed (Fig. 2) that the major route of excretion of cadmium after injection of chelating agents was in the faeces. The control group (group 3) excreted about 6% and 0.06% of the administered dose in faeces and urine, respectively, during the experimental 2 weeks. The group injected with BAL (group 1) excreted 28% in faeces and 1.4% in urine, while the group injected with a combination of BAL and DTPA (group 2) excreted 34% in faeces and 4.5% in urine. These results also suggested that the cadmium excreted through the bile after injection of BAL is not reabsorbed from the gut and is excreted in the faeces. Injection of DTPA with BAL increased both faecal and urinary excretion of cadmium.

The amount of cadmium in both liver and kidneys was significantly reduced after treatment with BAL or BAL and DTPA when compared with untreated rats (Table 1). The effect of BAL was more marked in the liver than in the kidneys. However, the mobilization of cadmium from the liver by BAL or BAL and DTPA together did not result in increased renal accumulation of the metal. In previous studies^{8-11,17}, when BAL was injected into animals before all the cadmium was bound to metallothionein, a major portion of cadmium associated with other bioligands was mobilized to the kidneys, increasing their content of cadmium. Thus the presence of metallothionein in the tissue may be an important factor in the effective therapeutic chelation of cadmium.

Our results provide the first evidence that BAL can be used successfully in proper conditions and at appropriate doses to mobilize cadmium from the liver without affecting its deposition in the kidneys, the critical organs in chronic cadmium toxicity. In reports from other laboratories^{19,20} of chelation therapy for cadmium poisoning, chelating agents were administered within

Table 1 Tissue content of cadmium in rats injected with CdCl_2 and treated with saline or BAL and DTPA

Group	Concentration of Cd (μg mean $\pm$ s.d.)	
	Liver	Kidney
1	2.95 $\pm$ 0.4*	3.85 $\pm$ 0.3
2	1.55 $\pm$ 0.5*	2.10 $\pm$ 0.1*
3	9.30 $\pm$ 0.4	5.15 $\pm$ 0.6

All rats were injected with $^{109}\text{CdCl}_2$ (1 mg per kg; 5 μCi). Three days later they were injected i.p. with BAL (50 mg per kg, group 1) or BAL and DTPA (50 mg per kg, group 2) or saline (group 3) for 2 weeks. Rats were killed 2 d after the last injection and tissue concentration of cadmium was determined from radioactivity.

* $P < 0.01$.

a short time of exposure to cadmium and the effect was measured by the rate survival of the animals. Those studies did not involve measurement of cadmium, and it was not clear whether the administered cadmium was either chelated or excreted from the body. One such study was later found to be invalid^{21,22} and retracted²³. Moreover, those studies do not have any relevance to the treatment of chronic poisoning. Our study reveals the potential use of BAL or BAL and DTPA together as a means of chelation of cadmium to remove it from the body without affecting the kidneys.

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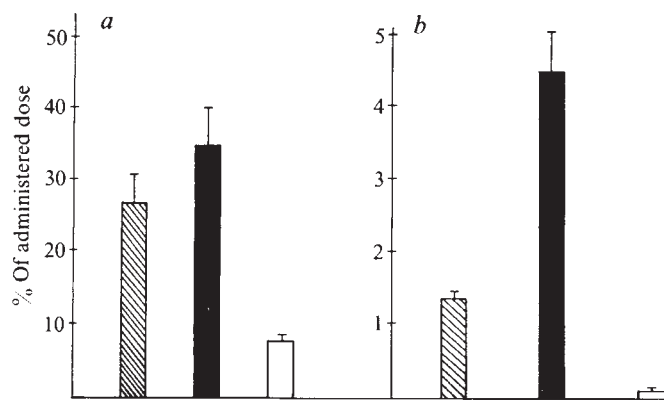
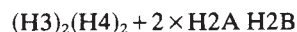


Fig. 2 Cumulative faecal (a) and urinary (b) excretion of cadmium in rats injected with CdCl_2 and treated with BAL (group 1, hatched columns) or BAL and DTPA (group 2, solid columns) or saline (group 3, open columns). Results are expressed as mean $\pm$ s.d.

Errata

In the article 'A low resolution structure for the histone core of the nucleosome' by A. Klug, D. Rhodes, J. Smith, J. T. Finch and J. O. Thomas, *Nature* **287**, 509-516, the third line of the equation displayed on the right-hand column of p. 514 should read



Also, in the same article, line 2 of the legend to Figure 7 should read 110° in place of 115° and 90° in place of 95°.

BOOK REVIEWS

The point of acupuncture

Patrick D. Wall

Celestial Lancets: A History and Rationale of Acupuncture and Moxa. By Lui Gwei-Djen and Joseph Needham. Pp.427. (Cambridge University Press: 1980.) £45, \$97.50.

THIS extraordinary book is a syncopated side step in the long march of Joseph Needham's writing of *Science and Civilisation in China*. He has completed five of the seven volumes planned, but so widespread is the current interest in acupuncture that this book is issued as a footnote to the next volume on the life sciences. I think it is also related to the final volume on the social background because one of the crucial questions for me is why it is that acupuncture has so suddenly emerged into the Western daylight. As this book shows, it has been practised for 2,500 years in China and it is no fresh news to the West. Extensive accounts were published in Europe by de Bondt in 1658, Cleyer in 1682 and ten Rhijne in 1683, and we can enjoy here the beautiful illustrations of their books. These early reporters who took part in the Dutch drive to the East were as enthusiastic as those *Time* magazine journalists who went with Nixon on his expedition to China: "The results with acupuncture in Japan which I will relate surpass even miracles", wrote de Bondt.

This raises a double question: why has acupuncture suddenly reappeared in the West, and how could it be that the West forgot for 300 years that all sorts of awful diseases could be abolished by simply sticking needles into the skin?

I believe there are three answers to the first question. The Chinese themselves swung away from their own traditional medicine in the last century and attempted with hopelessly inadequate numbers of doctors to introduce Western medicine. After the liberation of 1948, faced with the atrocious state of the medical services, all available resources were mobilized and traditional and Western practitioners were brought together. For the first time, surgeons and acupuncturists cooperated and the startling practice of major surgery under acupuncture emerged. Second, the Western media, searching for something good to say about China, latched on to the "miracle" since they could hardly praise communism. Third, there are many in the West, disillusioned with our ways of

thought, and with science in general and medicine in particular, who were delighted to accept that the pompous and ponderous academic establishment had been missing the point.

This book is divided into three parts. It starts by detailing the practice of acupuncture in the various schools with their historical developments and rationales. It is not intended as a clinical textbook but relates very clearly what is done and what were the ways of thinking about the body and disease. It also describes the less well-known treatment of moxa where the dried herb *Artemisia* is burned at the same points used for needling. This practice is also old and is widespread in many cultures, especially in the Arab and African worlds where localized cautery is used for the treatment of a wide variety of conditions from deafness to diarrhoea. The second part deals with physiological interpretations where an attempt is made to bring the old practices and explanations into an understandable framework in terms of modern physiology. Lastly, there is a section on the influences of other cultures which again emphasizes that the genius of China was never held in isolation

for long periods. It dominated Korea, Vietnam and Japan, merged with Russian thought over a huge area and influenced the successive waves of Western colonization.

Turning now to my second question of why acupuncture so strangely appears and disappears in the West, one must obviously ask first if it works. On this question, this enormously scholarly and careful book is curiously silent. Statistics are given for surgical anaesthesia which are impressive and coincide approximately with the observations of many Western teams of specialist observers. What is not mentioned is that these figures are taken from a highly selected group of patients and that many of these had been given powerful additional medication.

We are faced here with a real but rare phenomenon not to be explained by the simple action of physiological mechanisms which we all possess in common. A fairly frequent pain complaint associated with tenderpoints in muscles, the myofascial syndrome, does seem to respond to needling of these spots, and it is interesting that a number of the acupuncture points for pain coincide with the common sites of

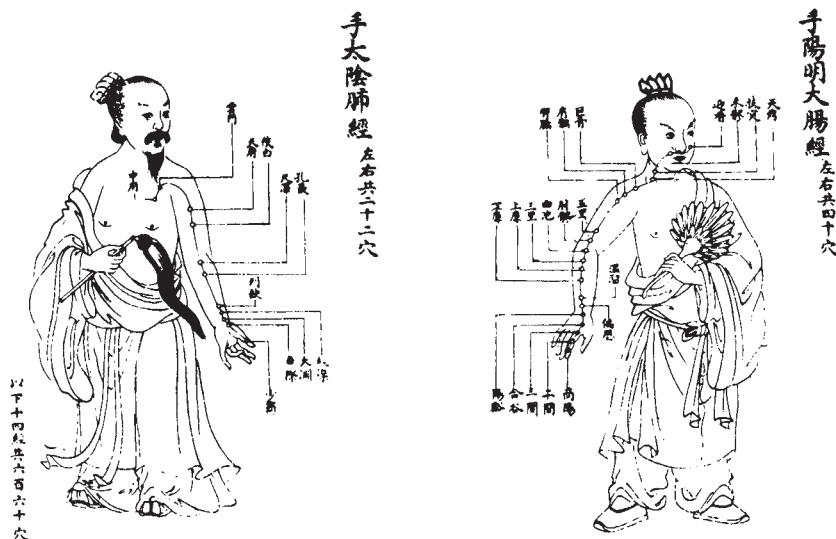


Veterinary acupuncture: points on the body of the horse. A drawing from the *Ma Niu I Fang* of +1399.

these tender points. For other types of pain, the results of only two clinical trials have been published and they are both negative. Other disease treatments have not been subject to anything but a creeping barrage of anecdote.

Before attempting an explanation, we badly need clear statements of what it is we have to explain. Acupuncture is liable to disappear from sight once more, under a heap of dissatisfied patients and impatient doctors: I hope we will not have to await its next renaissance before it is put to properly conducted trials which specify where and when it is effective. I hope this book with its careful description of "what" and "why" will encourage others to carry out those tests. □

Patrick D. Wall is Professor of Anatomy at University College, London, and co-author of the gate control theory of pain which is used widely in this book as an "explanation" of acupuncture.



The Cheirotelic pulmonic Thai-Yin acu-tract (left) and the Cheirogenic crasso-intestinal Yang-Ming acu-tract from Chang Chieh-Pin's *Lei Ching* of +1624.

The fundamentals of chemical kinetics

C. F. Wells

Basic Chemical Kinetics. By H. Eyring, S.H. Lin and S.M. Lin. Pp.504. (Wiley-Interscience: 1980.) £19.15, \$43.75.

I AM usually somewhat wary when I pick up a book with the word "basic" in the title, as I expect to find a highly simplified examination of the subject often without any pretence of rigour. This is very far from the case here, where "rigorous-fundamental" should be read for "basic".

After a brief introduction to rate equations and their integration, the authors devote their next chapter to potential energy surfaces. The dynamics of collisional processes are then considered, both from classical and quantum mechan-

ical points of view. This is followed by a treatment of transition state theory, a theory to which Eyring has contributed so much, and of the theories of unimolecular reactions in the gas phase and their application to some experimental data. After a similar discussion of bimolecular reactions in the gas phase, the authors concentrate on photochemical reactions in condensed phases as well as in the gas phase. In the final chapter selected topics concerning kinetics in condensed phases are discussed. Four appendices complete the book: three devoted to an elaboration of mathematical treatments used earlier in the book, the fourth containing 79 problems covering aspects raised in all the chapters.

In one sense, this book is an extension and up-dating of the classic text, *The Theory of Rate Processes*, written by Eyring in collaboration with S. Glasstone and K. J. Laidler, and first published in 1941 by McGraw-Hill. This new book, too, should be of interest to all (particularly

final-year undergraduates and research students) involved with the fundamentals of chemical kinetics. It will be invaluable to gas kineticists and to those concerned with photochemical kinetics, but perhaps has less direct application to the problems currently engaging solution kineticists. Chemists without an adequate mathematical background may find it hard going, and in this respect there are instances of undue compression of the text; for example l'Hôpital's Rule is referred to several times in Chapter 1 and is used in a problem in Appendix 4, yet nowhere is the Rule stated or defined.

Despite this reservation, the book is thoroughly recommended to all interested in the fundamentals of kinetic processes; it may well achieve "classic" status again for Eyring. □

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The sinister phenomenon

Freda Newcombe

Neuropsychology of Left-handedness. Perspectives in Neurolinguistics and Psycholinguistics. Edited by J. Herron. Pp.357. (Academic: 1980.) \$24.50, £13.80.

DEPARTURE from the usual pattern of right-handedness has often provoked annoyance, if not frank hostility, in the layman and has not inspired much in the way of scientific research until quite recently. There were occasional bursts of

interest, ranging from a brief and hesitant question in a nineteenth-century anthropological journal ("is my parrot left-handed?") to the more unequivocal pronouncement of the venerable Dr Henschen on sighting the asymmetrical skull of a gorilla in University College Museum: "this gorilla was probably left-handed".

But these flashes in the dark, with their interesting implications of paw preference and morphological correlates, were not followed up by phenomenological studies or biological speculations until clinicians

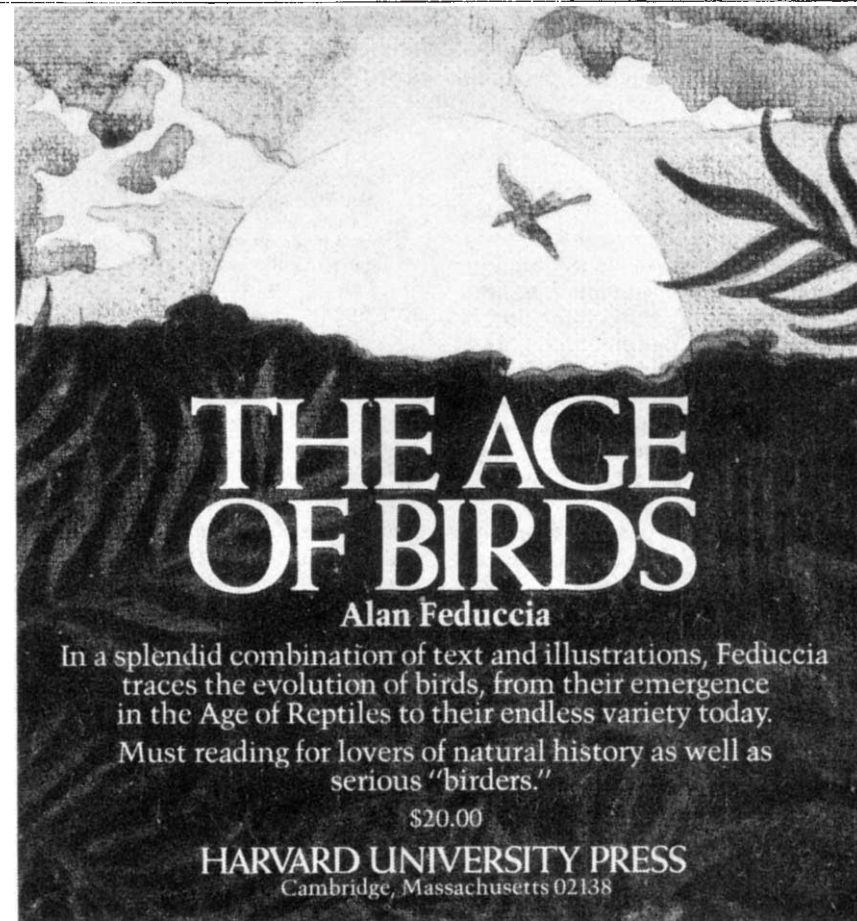
started to record anomalies of brain organization: speech disturbances due to damage to the right hemisphere of the brain, sometimes in left-handed patients. The work of Cheshier, Penfield and Roberts, Subirana, and Luria developed the theme that the link between brain organization and handedness is less predictable in the non-right-handed members of the population: whereas some 98% of right-handed persons have speech functions represented in the left hemisphere, the proportion of left-handed people exhibiting this pattern is in the region of 60–66%. The remainder show the reverse pattern except for a very small minority that show bilateral represen-

tation. It is also suggested that a tendency to bilateral speech representation in the left-handed might be advantageous, in that the prognosis for recovery of speech after CNS damage may be better than that for the right-handed patient.

Despite this knowledge — provided both by neurological patients and by very large populations of ex-servicemen with focal brain-wounds — received wisdom and cultural prejudice are hard to eliminate. There are still members of the medical and teaching professions who believe that the functional organization of the brain of all left-handed people is the mirror image of that of the right-handed. Left-handedness, mixed handedness and 'crossed lateralization' have also become popular 'explanations' of such developmental disorders as dyslexia, stuttering and delayed language acquisition. Small-scale studies, based on biased clinical samples, appeared to buttress this notion until the more comprehensive investigations of Rutter, Tizard and Whitmore (*Education, Health and Behaviour*. Longman: 1970), and of Margaret Clark (*Reading Difficulties in School*. Penguin: 1970) cast doubt on its validity. It was clearly time to question the flimsy evidence available and to collect some hard data. Many neuropsychological studies in the past two decades have addressed this issue and most of them are at least referenced in this interesting book.

The chapters cover a wide range — historical, embryological and genetic, and experimental. Harris has a delightful contribution on the topic of early theories, facts and fancies; he has done the reader signal service in distilling the essence of an early literature that few of us have the time or energy to cultivate. Studies of neuro-anatomical asymmetry are concisely reviewed by Witelson. There is an incisive critique of genetic models by Corballis, who accepts that of Annett as the most plausible; his conclusion that there is no convincing evidence to suggest that variations from the pattern of right-handedness and left cerebral control of speech 'are due to anything other than environmental causes' is less compelling.

There are other contributions that illustrate the two strategies that determine much applied research in this area: the data-driven approach and the model-inspired design. The former has produced a quantity of perplexing material but at least illustrates a complex background to interpretation that must take into account both sex differences and developmental changes in hemispheric organization; studies by Herron, Kinsbourne and Kocel are apposite. Model-making is serving its heuristic purpose. Satz's theory of pathological left-handedness and Levy's biological and genetic speculations cannot be lightly dismissed although their empirical consequences lack — as yet — the support of consistent replication and large-



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scale studies. The search for a definitive genetic model must continue; and chapters by Boklage and by Springer and Searleman on embryological factors provide an important stimulus in this direction.

We continue to be handicapped by the impure quality of the data. 'Familial sinistrality' is probably an important variable but it is seldom defined in a way that permits the formulation of a rigorous genetic theory. Sample sizes are often inadequate and the criteria of handedness vary from study to study. For example, 'sighting' has been used as an index in this book despite Annett's evidence that one third of a large, right-handed 'normal' sample used the left eye for sighting.

This book does a service in fairly representing current work and thinking in the field. It reminds us that the programming of handedness may be sought in the embryo, that non-right handers are not a homogenous group, and that patterns of hand preference must be studied in the light of developmental change and gender differences. It provides a good source of reference material and it contains a reasonable supply of ideas for future research. It is not a definitive work but it is compulsory reading for neuroscientists with either theoretical or practical commitments in this field. □

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The inner alarm clock

Arthur T. Winfree

The Timing of Sleep and Wakefulness: On the Substructure and Dynamics of the Circadian Pacemakers Underlying the Wake-Sleep Cycle. Studies of Brain Function, Vol. 3. By J.T. Enright. Pp. 263. (Springer-Verlag: 1980.) Flexi DM 49, \$29.

THERE is a growing conviction among "clocks" physiologists that circadian behavioural rhythms in animals are generated in the central nervous system. It is also widely believed that in most organisms many different tissues independently determine circadian fluctuations of their individual function, and that the mechanisms may reside even in single cells. Add to this the astonishingly precise timing of some circadian rhythms, in the order of 3 minutes in a 23½-hour cycle for example, and you have arrived at J.T. Enright's stepping-off point for this monograph — the idea that thousands of nerve cells function as separate circadian "pacers" whose collective rhythmicity disciplines each one to enhanced regularity.

This is an old idea, going back at least to the first big "clocks" symposium at Cold

Spring Harbor in 1960. By 1970 the theoretical literature contained elaborate electronic mockups, computer simulations and mathematical analyses of such ensembles of mutually synchronizing, non-linear oscillators. What Enright ably brings to the discussion in 1980 is a detailed computer simulation, complete with noise statistics, built along lines suggested by his concept of pacemaker neurones. His "coupled stochastic systems" model, nicely summarized in Chapter 16, is distinguished from its forerunners by a curious feature: only the longer-period pacers actually synchronize; the others go their merry ways but in so doing provide a pool of potentially entrainable pacers whose contributions become important when the ensemble is exposed to stimuli that affect phases and periods. From this unique feature many of the observed peculiarities of vertebrate circadian rhythms seem to follow, but the extent to

which they do so depends on a variety of adjustable quantities in the model. Enright is well aware of the implications of this:

The weary reader may . . . begin to suspect that the model system under consideration shows embryonic symptoms of developing into an amorphous monster: one which can, like an amoeba, engulf and account for any set of data by modification of one parameter or another, but which thereby merely demonstrates its lack of backbone. Can any model with so many parameters lay legitimate claim to being a simple model?

He further argues that explanations that are simple in the sense of elementary physics simply would not be plausible in context of "the workshop of natural selection", which seems more likely to "assemble a reliable pacemaker out of components which are sloppy".

The reader will undoubtedly wonder whether sleep and wakefulness are really the same as rest and activity; whether

circadian clocks really do function like pacemaker neurones, but a million times slower; and whether single cells do serve as independent clocks. But Enright's scepticism of his own model leaves little room for innovation by additional critics. Chapters 13 and 14 offer precise predictions for the outcome of feasible experiments which, if not fulfilled, will demand rejection or radical revision of the model. This detailed repartee between observation and interpretation is an edifying grace of Enright's monograph. In it we were treated to a thorough exposition of the present generalizations arising out of circadian physiology; and to a detailed exploration of the consequences of Enright's particular hypothesis.

What then is the upshot? Enright's model, based on observation of rest/activity cycles in birds and rodents, accounts for many of their peculiarities without having to resort to too many out-and-out *ad hoc* suppositions. It gives a reasonable vision of the kinds of mutual interaction among the pacers that would enhance their precision to the levels sometimes observed.

However, I suspect that much of the fitting to phase-resetting data and period-changing data could be managed with equal elegance by rather different models; there exists at present little of a factual nature to distinguish alternative visions of the internal organization of the central nervous system clock. Yet here we encounter what, from my present viewpoint among models of human circadian rhythms, seems a primary value of Enright's contribution. Research on sleep/wake rhythms is now in the midst of an explosive blossoming of discovery. Enright's model correlates definite facts and makes distinct critical predictions. This is just what we need, alongside the alternative visions of Wever, Kronauer and others, to catalyse the asking of sharp experimental questions.

Like almost all models in the behavioural sciences, Enright's is partly a fairy tale — in this case, a pleasing one, done with a sense of humour, but not frivolously. He remarks on p.249 that "... there is not the least doubt in my mind that the models as formulated will prove to be wrong; wrong because they are oversimplified; wrong because they have ignored one critical process or another; and wrong because some elements of the models arise from mistaken points of departure". But as Francis Bacon remarks in his *Novum Organum*, "truth will sooner come from error than from confusion". In my view, debate over Enright's facts and interpretations could take us a long way toward understanding one of the most conspicuous facts of human experience, the rhythm of sleep and wakefulness. □

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With reference to variable stars

R. J. Tayler

Theory of Stellar Pulsation. By J. P. Cox. Pp. 380. (Princeton University Press: 1980.) Hardback £21.90, \$50; paperback £7.50, \$17.

FOR some time there has been a need for a new book on the theory of variable stars to consider the considerable developments in the subject that have taken place since the publication of Rosseland's *The Pulsation Theory of Variable Stars* (Oxford University Press, 1949). Now there are two new books: *Nonradial Oscillations of Stars* (Tokyo University, 1979) by Unno, Osaki, Ando and Shibahashi, which does not discuss the more widely studied and better understood radial oscillations, and the present book which covers the whole subject. J. P. Cox has written many review articles on variable stars and this, the book which has grown out of them, provides a thorough survey both of the foundations of the subject and of recent developments.

In 1949 it was clear that many intrinsic variable stars were pulsating radially, but important questions remained relating to the physical cause of the instability exciting the oscillation, to the observed phase relationships between light and velocity

variations, to the processes limiting the instability to finite amplitude and to the evolutionary status of variable stars. The answers to all except the third of these questions are now reasonably clear. However, there is still considerable difficulty in deciding whether a star with a deep convective envelope will pulsate, because there is no good description of time-dependent convection, and there are arguments about whether the stellar masses required to give the correct pulsations agree with those implied by stellar evolution theory. Although there are clues to what produces the finite amplitude limitation of the instability, it is difficult to study the full non-linear problem without crude approximation. The study of non-radial oscillation is much more difficult than that of radial pulsation, but it is clear that non-radial oscillations are present in some stars and, indeed, convection is a non-radial oscillation of very high order. The study of non-radial oscillations has recently been stimulated by observations of small fluctuations in the properties of the Sun.

Cox gives a clear and detailed account of the subject, and this book is a very welcome addition to the literature. If I have any major criticism, it is that it is perhaps too detailed to be used as a textbook; for that purpose I would have omitted some references to topics which could not be discussed in detail. It will, however, be invaluable as a reference book. In particular, it contains a list of relevant papers which is more than 30 pages long. □

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The British Museum (Natural History), in collaboration with John Wiley, have just published *Animal Identification: A Reference Guide* in three volumes. Each volume provides a listing of primary reference sources — Vol. 1 to marine and brackish water animals, Vol. 2 to land and freshwater animals (excluding insects), and Vol. 3 to insects — to assist non-specialists in identifying any animal. The three-volume set costs £31.

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